

Gorbachev needs an answer

Mr Mikhail Gorbachev's visit to London last week has sharpened still further the issue of NATO's tactical nuclear weapons. NATO cannot pretend that the issue is technical and exclusively its own concern.

LAST year's tragic earthquake in Armenia has cast a long shadow. That is the simplest explanation why Mr Mikhail Gorbachev's visit to London last week was probably as much a disappointment for him as for many of those who heard what he had to say. His original plan had been to follow his speech at the United Nations in December, when he made public his plan unilaterally to cull 500,000 people from the Soviet armed services, by travelling to Cuba and then to London. Then, in the interregnum between the Reagan and Bush administrations in Washington, it would have been easier for him to open a fruitful dialogue on arms control. By last week, the momentum had been dissipated. Worse, the British government, now preoccupied with the sharpening dispute within the North Atlantic Treaty Organization (NATO) about the continuing role of nuclear weapons in Europe, was in no mood to respond imaginatively.

The issue is simple, but none the less corrosive on that account. The treaty on missiles of intermediate range (INF) signed at the end of 1987 regulates the removal from Europe of missiles capable of carrying nuclear warheads a distance of 1,500 km or more, but only implicitly regulates missiles of shorter range. (A precondition of INF was the readiness of NATO and the Warsaw Pact to remove tactical nuclear weapons from the scene.) Now, NATO wishes to improve ("modernize") the performance of its tactical nuclear weapons over the next few years. Specifically, there is a plan for replacing the Lance missiles now sited in West Germany with improved rockets capable of travelling 500 km. The Soviet Union disapproves — Gorbachev said so plainly last Friday. So, for different reasons, does West Germany.

Gorbachev's view is that improving the performance of NATO's tactical weapons will break the spirit if not the letter of INF. He said last week that if NATO goes ahead with its planned modernization, the talks on the regulation of conventional arms now under way in Vienna will be jeopardized. NATO's counter-argument is that nuclear weapons remain a necessary part of the defence of Western Europe. At least so long as the intended agreement on conventional forces in Europe remains in the future, nuclear weapons may be the only way of halting an armed incursion. That view is not new: NATO has held to the same doctrine for a quarter of a century. The novelty is twofold — there is now a chance of winning an agreement on conventional forces, while West German voters

and thus, perforce, the West German government are increasingly attracted by the even brighter prospect of a more general disappearance of political and military tension in Europe.

Formally, so far as NATO is concerned, the issue is intended to be settled in the summer of this year, after the Bush administration's review of its security interests (and the associated defence budget) is complete. But that is more than NATO's managers can reasonably expect. If anything, the reluctance of West German voters to support governments which agree to give house-room to modernized weapons is more likely to grow than to melt away. A further difficulty is that the Vienna negotiations will not make sense if aircraft and other remote means of delivering bombs (nuclear or otherwise) are excluded, which is what NATO asks.

That is why the need now is for a constructive compromise. Gorbachev's protest that NATO's insistence on improved nuclear weapons in Europe would jeopardize the Vienna negotiations was quickly countered, in London and Washington, by a simple reiteration of the need to modernize outdated nuclear weapons. Why not, instead, acknowledge that the need for battlefield nuclear weapons in Europe would be diminished if the Vienna talks succeed, and thus make the planned changes contingent on failure at Vienna? That way, Gorbachev and his counterparts in the West could be saying much the same thing, while the important negotiation at Vienna would be invested with a sense of urgency. □

Disorderly publication

There is no reason why discoveries should not first be published in daily newspapers, but there are drawbacks.

THE great fuss about cold fusion over the past few weeks raises important questions about the scientific literature and its function to which the scientific community should give urgent thought. Reports that nuclear fusion had been brought about in an electrochemical cell first appeared in two financial newspapers — the *Wall Street Journal* and the *Financial Times* — on 23 March. Professors Martin Fleischmann (Southampton) and Stanley Pons (Utah) were reported to have accomplished fusion in an electrolytic cell.

Soon it also became known that a group at Brigham



Young University led by Professor Steven E. Jones was ready with comparable but not identical observations. The University of Utah promptly held a press conference at which, among other things, an article by Fleischmann and Pons was said to have been submitted to *Nature*. In the event, the first article to arrive was from the Brigham Young group, but one from Fleischmann and Pons reached us later; an extended version of it was published last week under the heading "preliminary note" in the *Journal of Electroanalytical Chemistry* (see page 537).

It is no disrespect to any of those concerned to compare the dilemma created for *Nature* by these events to that occasioned a year ago by the article in which Professor Jacques Benveniste and colleagues claimed that indefinitely diluted reagents retain their biological effectiveness. The claim flies in the face of orthodox belief, but the data available are insufficient for a careful judgement of its validity. But on this occasion, *Nature* has followed standard procedures. Both articles have been sent to referees, each is now being revised in the light of the many comments that have been made. Many of these would normally require extensive reinvestigation. In the normal course of events, weeks or months might go by. Researchers are used to delays of that kind, especially when they have important things to say. The process is beneficial because it improves the quality and reliability of what is published.

What is to happen now? Public and professional curiosity now require rapid publication, but there is only a small chance that either group of authors can make all the amendments asked of them in a short time. So should publication be postponed until they can do so? There is a sense in which that would not matter: the information is already in circulation. But publication is more than merely the circulation of information to those with access to the appropriate networks. General availability is crucial. That is why revised versions of one or other or of both articles will be published later in the month. In these exceptional circumstances, they will be accompanied by such comments of the referees as remain pertinent.

None of this implies that the peer-review system is infallible. Those who live with it know that it is shot through with imperfections. There is, for example, a danger that it induces too much uniformity in the literature. But the system is a powerful means by which good ideas are made better on their way into print. The practical question for the scientific community, and for journals in particular, is to adapt the system (and the process of publication) more swiftly to the steady improvement of communications.

Nobody can sensibly complain about those developments. But there remains an immense difference between the discussions thus stimulated within the scientific community and the broadcasting of claims not fully tested. Fleischmann and Pons said at their press conference that their work was in danger of "leaking out", much as Benveniste last year cited reports in *Le Monde* as a cause for urgency. But the authors of an experiment are best

placed to determine when and how news of their work is published generally. It is naturally difficult to bottle up exciting news, but impatience is a poor guide to action. The greater the importance of a discovery seems, the longer it should be worthwhile waiting to see it properly established. □

Pan-European university?

The Swiss are nursing the idea of creating a European university and should be encouraged.

PLANS to turn the European Economic Community (EEC) into a genuine common market at the end of 1992 may not yet have done much to change economic behaviour in the 12 countries directly involved, but they have had a powerful influence on outsiders. Japanese and US companies are busy building factories in member states or forming business partnerships with companies registered there. Governments are similarly impelled by a sense of exclusion to seek membership of the EEC: Turkey has formally applied, Austria says it will and Norway (for the second time) is brooding. The way things are going, by 1992 there may be only one European outsider left outside — Switzerland.

This prospect keeps stoical Swiss awake at nights. Their difficulty is plain: the constitution of the Swiss Federation, with its devolution of power from the centre, could not be changed without changing Switzerland. Yet it was already plain last year (see *Nature* 336, 323 *et seq.*; 1988) that anxiety about the emerging single market had become an influence in Swiss planning of research as well as a source of fear that Switzerland may become an amalgam of holiday resort and retirement home for nationals who choose to spend their working lives elsewhere. But anxiety is a catalyst of the imagination, whence the notion now gaining ground that Switzerland, within the confines of its constitution, might make a distinctive contribution to the development of Europe by creating a university on a grand European scale.

This is a daring notion, but none the worse for that. Europe, taken as a whole, has a commendable diversity of universities, but most of them are bound to seem parochial by the yardsticks of the pan-Europeans. But Switzerland has a cosmopolitan tradition rivalled only by that of the Netherlands and might — so the calculation goes — be able to build from its existing institutions a distinctively European university that can be counted among the half dozen or so outstanding universities in the world. For the time being, of course, this is just a dream, but one which many influential Swiss are eager to explore. Altruism is not the only driving-force, of course: to be the site of an acknowledged leader among European universities would be an assurance against isolation, while a strong research programme would undoubtedly engender spin-off. But it is a notion that universities elsewhere in Europe should be ready to welcome. □

Prospect of achieving cold fusion tantalizes

- Confirmation reports trickle in
- Dispute over primacy

Washington

SCIENCE by press conference continued this week as Texas A & M University at College Station announced on Monday that researchers there had replicated at least one feature of experiments purporting to show nuclear fusion at room temperature. By the afternoon, a similar announcement had been put out by the press office at the Georgia Institute of Technology, Atlanta.

The Texas group, led by Charles Martin, is getting between 60 and 80 per cent more energy out of its experimental apparatus than it is putting in.

The Georgia group, led by Dr James Mahaffey, has measured neutrons from a palladium cell, recording a fifteenfold increase above background when current was flowing through the cell.

University of Utah chemist Stanley Pons, who first described the cold-fusion experiments at a press conference on 23 March, has confidently predicted that several groups would confirm his experiments. Other groups in the United States, working from preprints of a paper by Pons and Martin Fleischmann that has now appeared in the 10 March issue of the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* (261, No. 2a, 301-308; 1989), have not reported either the excess heat production or the appearance of 2.5 MeV neutrons.

Meanwhile, a legal battle is shaping up between the University of Utah and Brigham Young University (BYU) over who first discovered the cold-fusion process. The University of Utah filed a patent application during the week of 13 March, but officials at BYU believe that research done by faculty member Robert Jones along similar lines clearly precedes the work done by Pons. BYU will file a patent application shortly.

Paul Richards, director of public communications at BYU, says his institution felt no pressing need to obtain a patent, as applications for the technology were likely to be far in the future, and the results were of more scientific than commercial interest. Richards adds that BYU felt compelled to file a patent claim in response to accusations that Jones had stolen Pons's ideas.

The relationship between the BYU and University of Utah research teams has been rocky for some time. Last September, Jones was asked to review a grant application submitted to the Department of Energy (DoE) describing the Utah

team's efforts in cold fusion. The grant was ultimately approved. But as well as submitting his comments on the grant to DoE, Jones asked that DoE contact Pons and suggest a collaboration using a neutron detector Jones had developed. DoE did so, but efforts to bring the two groups together were unsuccessful. Ultimately the relationship between the two teams deteriorated to the point where the presidents of both universities met with the leaders of the two research teams to try to clear the air.

That meeting took place on 6 March. Both groups agreed to meet on 24 March at Salt Lake City airport to send simultaneous express packages to *Nature* containing their research results. The timing was crucial, as Jones had agreed to speak at a meeting of the American Physical Society in early May, and they were anxious to have a paper accepted by a peer-reviewed journal before then. Jones cancelled a seminar on his work that had been arranged for 11 March.

But in the intervening weeks, the University of Utah did two things that BYU considered in violation of the agreements worked out on 6 March. On 11 March, Pons and Fleischmann submitted a paper containing details of their cold-fusion experiments to the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, and on 23 March held a press conference to describe their findings. The BYU team was particularly annoyed when, during the press conference, University of Utah vice president for research, James Brophy, told reporters he was unaware of any other groups doing similar work.

The BYU group decided there was no longer any reason to delay, and sent its paper to *Nature* on 23 March. That at the University of Utah sent its on 24 March.

Brophy says the press conference was scheduled after the university learned that a local reporter was planning to publish a news item describing the findings. But Pons says the press conference was held because the paper had already been accepted by the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, although a news release from the journal's publisher, Elsevier Sequoia, states that the paper was accepted on 30 March.

Meanwhile, in Utah, the state legislature has set aside \$5 million to support fusion research once a state commission to be appointed by the governor is convinced that the findings have been confirmed. □

SOVIET SUBMARINES

Pollution fears for Norwegian Sea

London

A Soviet nuclear submarine of experimental design sank last week, with the loss of 42 lives, in international waters in the Norwegian Sea, between Bear Island and the Norwegian coast.

The immediate reaction in Norway has been one of consternation, even though Dr Willy Ostreng of the Fridtjof Nansen Institute (which studies security issues in northern waters) says that it "was only a matter of time" before such an accident happened in one of the world's most "heavily submarine-infested" channels.

Surface water and air samples collected by the Norwegian authorities have so far shown no signs of radioactive contamination. A Norwegian team is expected to reach the area on Thursday this week, with the intention of collecting deep-water samples, when it is hoped that a preliminary estimate of the immediate danger of contamination will be possible.

The submarine was equipped with two reactors, each of them cooled by a molten mix of lead and bismuth. If the fire which appears to have been the immediate cause of the disaster did not damage the integrity of the reactor compartments, and if the reactors were shut down before the ship sank, there is at least a chance that radioactive fuel elements will be entombed in a now-solid mass of metal from which radioactive material will be released only slowly.

As after the Chernobyl accident, the incident has become something of a test of *glasnost*. The Soviet side has released a certain amount of information, but *Pravda* complained at the weekend that Western journalists were able to get more information from Soviet embassies abroad than its own correspondents were able to gather in Moscow. Even so, the Norwegians are still clamouring for technical details so as to be able to calculate the long-term risk.

Vera Rich

SOVIET UNION

Reforms begin to take effect

London

THE Soviet Union announced last week that, under its new mental health legislation, more than 60,000 people have been taken off the registers of psychiatric clinics during the past year in Moscow alone. Eventually, it is expected that some 1.2 million Soviet citizens will be removed from the registers. How many names will remain on the registers is unclear. But the new legislation stresses that no citizen may now be hospitalized without his or her consent, except for people "posing an immediate threat to society".

Vera Rich

Culprit found at Cornell

Washington

BLAME for a computer 'worm' that crippled thousands of installations on the Internet computer network last November has been placed squarely on the shoulders of Robert Tappan Morris by a commission of inquiry at Cornell University, where Morris is a graduate student.

The attack raised many questions about the security of computer networks to unwarranted entry. But according to the Cornell inquiry, made public late last month, Morris's actions did not so much represent "an heroic event that pointed up the weaknesses of operating systems" as "a juvenile act that ignored the clear potential consequences".

The worm was released on 3 November, 1989 and infected computers using particular versions of the UNIX operating system (see *Nature* 336, 97; 1988). Although it was first diagnosed as a computer 'virus', the infecting program is more properly known as a worm, as it does not require another computer program to act as a 'host' to carry it from one machine

to another.

According to the inquiry's findings, the worm consisted of two parts; a 99-line 'probe' written in the 'C' programming language, and a much larger 'corpus' that had been compiled into machine language. If the probe was able to get into a computer, it sent for its larger body. The probe used four basic methods to gain entry. Two used specific design features of UNIX mail programs that allowed the worm to enter a new machine without passwords that would normally be required. Another avenue was essentially a sophisticated guessing game in which the worm exploited a human tendency to choose simple passwords related to account names. A fourth method exploited a design feature of UNIX that allows people to use the same password on several different machines where they have accounts.

After releasing the worm on the evening of 3 November, Morris evidently decided he had made a mistake, and asked an associate at Harvard to send a message to computer users around the country telling them how to protect themselves against the worm. But the warning was sent by a slow method, and arrived after others had discovered the worm and developed ways to defeat it.

The commission concluded that several thousand computers were infected by the worm, but thousands more had to be shut down as a precaution to prove that infection had not occurred.

The commission points out that Morris was able to exploit security flaws in the UNIX operating system but that UNIX was never designed to be a secure system and many of the flaws were already well known. The commission concluded that creating the worm required "dedication and perseverance rather than technical brilliance", adding that Morris "displayed naive conceit in assuming that he could launch an untested, unsimulated, complex worm onto a complex network and have it work correctly the first time".

Morris declined to be interviewed by the commission of inquiry on the advice of his counsel, but from talking with his colleagues and friends and by examining logs of Morris's computer activities, the commission concluded that Morris alone had written the worm.

Although the commission recommends strong disciplinary action, it suggests that the discipline "should not be so stern as to damage permanently the perpetrator's career". Cornell University has not yet decided on any punishment for Morris. An investigation of the incident by the Federal Bureau of Investigation has not yet resulted in any indictments.

Joseph Palca

Britain to expand?

London

BRITAIN has played only a minor role in Arctic research until now, with total funds from the research councils of little more than £1.5 million last year. The Natural Environmental Research Council (NERC) now wants to change this, so that Britain can act at least as a useful partner in international research programmes.

Last year, NERC spent less than £500,000 on Arctic research, mostly on individual research grants and training awards; there are no institutional research programmes devoted to the Arctic. NERC has now asked for an additional £2 million a year from next year's science budget for Arctic research.

The council has rejected the suggestion that it create an Arctic research institution with permanent research bases comparable in size to those in the Antarctic. "On political and financial grounds", this would be unrealistic, it says in its first strategy document for the Arctic, published this week. Less ambitiously, it wants to build a small base on Svalbard, the archipelago north of Norway. The base would be intended to house about 20 researchers and to cost only £70,000 to build and £50,000 a year to run.

The council also wants to increase support for the Scott Polar Research Institute at the University of Cambridge. With more staff and increased and secure long-term funds, this institute would become the focus of British Arctic research, says Dr John Bowman, secretary of the council. But he stresses that expansion here will not compromise research elsewhere in the country. Research areas to which the council gives priority are meteorology, solar-terrestrial physics, oceanography, glaciology, ecology, geology, socio-economic sciences and engineering.

Christine McGourty

SPACE SCIENCE

Explorers selected

Washington

The National Aeronautics and Space Administration (NASA) last week announced the first four in a series of small scientific missions that will be launched on existing expendable launch vehicles. The small scale missions — with total costs for each spacecraft expected to average \$30 million — are intended to provide research opportunities for a new generation of space scientists and engineers.

The four projects, chosen out of 51 applications, include the Solar, Anomalous and Magnetospheric Particle Explorer, the Submillimeter Wave Astronomy Satellite, the Fast Auroral Snapshot Explorer and a global ozone satellite that will carry a total ozone mapping spectrometer.

Joseph Palca

COMPUTERS

Pakistani virus invades Indian neighbour

New Delhi

'C-BRAN', a computer virus of Pakistani origin, has hit personal computers throughout India in what is thought to be the first epidemic of its kind in the nation. The incident highlights India's extensive trade in pirated software and copied floppy disks.

In just four weeks, 'C-bran' showed up in computer schools and a private company in Bangalore and in some 20 companies in Bombay, including an international bank. The virus was also reported from Madras, Kanpur and Vijayawada, a small town where 750 of 1,000 floppy disks used by a private company were found to be infected. Scientists at the Delhi University Computer Centre said their machines were invaded by the 'ashar' variant of the virus.

Dr N. C. Kalra of the Indian Institute of Technology in Delhi, who detected 'ashar' in the institute's computer, centre blamed the epidemic on pirated software. No virus has yet shown up in computers linked by electronic networks but government agencies have been alerted. India's NICNET links the Cybercomputer mainframe computer in the Planning Commission with portable computers in district headquarters while another network, ERNET links the computer centres of eight educational institutions. The six major commercial computer networks all claim to have strengthened their security system following the epidemic. K.S. Jayaraman

RAIN FORESTS

Disputes about destruction

São Paulo

By quoting figures that downplay the extent of Amazon deforestation, Brazil's President José Sarney last week touched off a dispute over estimates of rain-forest destruction and embarrassed his nation's own Instituto de Pesquisas Espaciais (INPE, Institute of Space Research).

Sarney's comments came as he signed into law legislation that creates the 'Nossa Natureza' (Our Nature) programme designed to protect the Amazon forests (see *Nature* 338, 286; 23 March 1989).

He harshly attacked 'foreign intervention' in Brazil's Amazon policy and, to prove that the destruction of Amazon rain forest is not as bad as foreigners claim, he noted that the latest data on deforestation calculated by INPE scientists show that just 5.12 per cent of the rain forest has been cleared since the colonization of Brazil began in 1500.

But that figure is in dispute. A 'green' federal deputy, social-democrat Fábio Feldmann from São Paulo, claimed the data had been 'manipulated' to suit government needs. The '5.12 per cent' derives from figures on the destruction of tropical moist forest. But the INPE team responsible for the analysis related the area destroyed (250,429 square kilometres) to the total area of the Brazilian Amazon region,

rather than to the total area of rain forest, which is smaller (see map).

Barbosa said the team did not relate deforestation data to total rain-forest area because "we do not know the true extent of the rain forest". But other INPE researchers said they thought it was a mistake to present the data that way. To pre-



The Brazilian Amazon is legally defined as consisting of the whole of six states (Acre (AC), Amazonas (AM), Rondônia (RO), Roraima (RR), Pará (PA) and Amapá (AP) and parts of three others (Mato Grosso (MT), Maranhão (MA) and Tocantins (TO)). Only about three quarters of its 4,906,784 square kilometres contains rain forest. The remainder consists of savannah.

CLIMATE

Worldwide atmosphere authority resisted

Paris

AN official advertisement in French newspapers setting out the declaration signed by 24 nations at last month's environment conference at the Hague (see *Nature* 338, 193; 1989) has provoked an angry reaction in Brazil. The advertisement appeared on Monday, 3 April in all 24 signatory countries, but the version in *Le Monde*, *Figaro* and *Libération*, published at a cost of FF1.8 million (\$290,000) to the prime minister's information service, reproduced the signatures of the 24 national representatives alongside an additional statement which, it seems, was not part of the declaration.

The statement says that the 24 signatories call for the creation of "a worldwide authority, invested with real decision-making and executive powers to save the atmosphere". The statement also says that the signatories are "ready to surrender part of their national sovereignty for the common good of humanity as a whole".

Paulo Tarso Flecha de Lima, the Brazilian diplomat whose signature appears in the advertisement, was not prepared to go this far at the Hague. The Brazilian government, criticized for destruction of its rain forests, has angrily resisted attempts by other nations to enforce a policy of conser-

vation. Brazilian President José Sarney recently accused the foreign media of conducting an "alarmist campaign" against Brazil and complained that developed countries were backing policies that would make Brazilians "slaves".

The United States, which did not send a representative to the Hague meeting, also has no enthusiasm for a new worldwide authority. At a congressional briefing last week, William A. Nitze, a US State Department representative, said it was "premature to be considering a 'law of the air' or supranational authorities to deal with climate change".

With European elections in full swing in France, concern for the environment has become a campaign issue. Opinion polls following a good result in recent municipal elections have shown the hitherto dormant French ecology party to be an emerging alternative centre force and both the incumbent socialists and the right-wing opposition have been scrambling to demonstrate their interest in environmental issues. That may partly explain the government's decision to advertise the Hague declaration with President François Mitterrand's signature prominently on display.

Peter Coles

pare the report for the president, a task force was rapidly assembled and a building made over to it. In a vain attempt to prevent leaks to the press, INPE even moved its press officers away from the remote-sensing team.

Recalculation of the INPE figures using an estimate of the total area of rain forest (3,700,000 square kilometres) suggests that 6.8 per cent of the rain forest has been destroyed. But that figure is still much smaller than the 12 per cent deforestation claimed by the World Bank in its report *Government Policies and Deforestation in Brazil's Amazon Region*, released in January.

That figure is based largely on projections from Landsat data published in 1986 by Philip Fearnside, a US scientist working at the National Institute of Amazon Research at Manaus. But Fearnside himself estimates Amazon deforestation at 8 per cent. INPE's director-general, Márcio Noqueira Barbosa, says his institute's estimate is more accurate because it is based on 1988 Landsat images.

Landsat images are received every day at INP's station in Cuiabá, Mato Grosso. INP remote-sensing director Roberto Pereira da Cunha's team used Landsat Thematic Mapper data, with 30-metre resolution in the visible and near-infrared regions, to compile its data on deforestation. Cunha, who is clearly shaken by the controversy caused by the data, says they have studied 234 images, of which 104 were selected for detailed interpretation because they contained deforested regions. He claims the research began in August 1988 but this too is disputed. Other scientists say work began in earnest only in late March this year.

Ricardo Bonalume Neto

SSC

India offers help

New Delhi

INDIA is proposing to participate in the US Superconducting Super Collider (SSC) project by contributing manpower and equipment but not money, according to Science Minister K. R. Narayanan. He told parliament last week that informal discussions had taken place between Indian and US scientists but "no formal agreement has been signed nor any commitment on expenditure agreed to".

Narayanan said India was joining the SSC project because it provided "a good opportunity for Indian scientists to work at the frontiers of science and technology".

A spokesman for the Indian team negotiating with the SSC project authorities confirmed an Indian offer of \$50 million in hardware, particularly particle detectors, but "there is not going to be any monetary contribution at all", he said.

K.S. Jayaraman

New sales tax hits science

Tokyo

JAPAN'S controversial new sales tax, which came into effect last week, has not only placed an additional burden on Japan's consumers but has also pushed up the costs of scientific research. The budgets for the science-related ministries have been increased over the past few months to cover some of the costs of the new tax, but universities and research institutes will still find themselves worse off.

The sales tax marks the most sweeping reform of Japan's tax system since the Second World War and has met almost universal opposition. Although set at a low overall rate of 3 per cent, the tax covers almost all forms of 'consumption'. Books, food, transportation, rent and even the costs of childbirth and tombstones are taxed. Scientific research is no exception.

Although the tax takes up only a small percentage of ministerial budgets, it does constitute a significant proportion of the increase in outlay for science between the fiscal years 1988 and 1989 — about 20 per cent, or ¥5,900 million (\$45 million), in the case of the Science and Technology Agency.

The tax is levied on the purchase of equipment, books and journals and also adds significantly to the cost of construction of new facilities. Although the various science-related ministries have made upward revisions in their budgets to cover the tax, some of the burden will inevitably have to be borne by the universities and research institutes.

For example, although the Ministry of Education, Science and Culture has raised the budget for fiscal year 1989 for grants-in-aid of research to cover the increased costs of equipment, the value of multi-year grants awarded before implementation of the tax (for example, the 'special distinguished grants' which run for 3–5 years) will remain unchanged.

The new tax has helped to make Noboru Takeshita the most unpopular prime minister in the post-war era. The latest polls show that less than 10 per cent of the public support his administration. And revelations last week that Takeshita received almost ¥100 million (\$760,000) in donations from the scandal-ridden Recruit company in the run-up to his election as head of the ruling Liberal Democratic Party (LDP) have further weakened his position.

Calls for Takeshita's resignation are growing within the LDP and he may not be able to survive in office for longer than a few more months. Under a new leader, the sales tax could be revised, but as it has taken the LDP ten years and two failed attempts to introduce it, the tax seems likely to remain.

David Swinbanks

More yen for joint research

Tokyo

LEGISLATION at present before Japan's Diet will allow the Science and Technology Agency to launch a new international programme in October to promote joint research with overseas institutions. The new programme will for the first time open a way for Japanese government money to be spent on research facilities in foreign countries.

The new research initiative will be modelled along the lines of the agency's Exploratory Research for Advanced Technology (ERATO) programme, which recently received high marks in an assessment by US researchers (see *Nature* 337, 196; 1989). ERATO, which is run by the agency's Research and Development Corporation of Japan (JRDC), finances 'high-risk' research with potential for application. Although some foreign scientists participate in ERATO, the research is carried out entirely within Japan. The new 'international ERATO' will allow participation by overseas research organizations (in government, industry and university) through cooperative research agreements. Legislation allowing JRDC to establish such agreements is expected to pass through the Diet within the next month or two, say agency officials.

The programme will support teams of about 20 researchers, about half based in Japan and half overseas, with funds of about \$8 to \$13 million for periods of 3 to 5 years. Agency officials hope that participating countries will contribute research funds and facilities as well as researchers, although it is possible that JRDC will 'rent' research laboratories overseas as the corporation now does in Japan for ERATO.

Until now, it has been almost impossible for Japanese government organizations to spend money on research facilities in foreign countries because of the lack of rules and mechanisms for auditing and accounting. A classic example is the bureaucratic delay encountered by Tokyo Astronomical Observatory in its attempts to build the world's largest telescope in Hawaii (see *Nature* 311, 5; 1984). After years of debate, the telescope has still to win government approval. By renting rather than buying research facilities, agency officials are confident they can avoid such problems.

Masahiko Noda of the agency's International Affairs Division says the programme received a favourable response in the United States last month when he visited various organizations, including the Office of Science and Technology Policy, the National Science Foundation, universities and non-profit research institutions. And Kaname Ikeda, director of the division, says a research project with the

United States could be set up within a few months using the new mechanisms created under the US–Japan Science and Technology Agreement signed last June.

But some difficulties may be encountered in trying to apply the ERATO model to the United States. ERATO projects are headed by senior Japanese scientists hand-picked by JRDC, an approach that runs counter to the open and competitive research system of the United States. And agency officials admit that they will probably have to adopt a more 'flexible' approach in foreign countries than that used in Japan.

Apart from international joint research projects, the new organization in JRDC will administer the agency's new post-doctoral fellowship scheme for foreign researchers which began last year (see *Nature* 335, 287; 1988), and will provide accommodation, Japanese language training, counselling and information for overseas researchers working in Japan. The agency has set aside ¥418 million (\$3.2 million) for the programme in the second half of fiscal year 1989 and about ¥800 million to build 50 homes for foreign researchers in Tsukuba Science City by 1991.

David Swinbanks

HIGHER EDUCATION

Vet schools reprieved in Britain

London

FEARS that two of Britain's six veterinary schools may have to close receded last week when the Universities Funding Council decided to ask the Ministry of Agriculture, Fisheries and Food (MAFF) to reconsider future manpower requirements in the veterinary profession. A review is expected to show that previous forecasts of manpower needs were too low and that all six schools are necessary.

A report published in January recommended the closure of the veterinary schools at the Universities of Glasgow and Cambridge and the redistribution of their staff to the remaining four schools (see *Nature* 338, 191, 16 March 1989). But the British Veterinary Association now argues that more students are needed and that earlier estimates it gave to MAFF were wrong.

The decision was welcomed by James Armour, head of the veterinary school at the University of Glasgow. But he remains dissatisfied that the recommendation to close Glasgow was partly based on its proximity to Edinburgh, where there is another veterinary school, rather than solely on academic performance.

Christine McGourty

ALASKA OIL SPILL

Fisheries first to suffer

Berkeley

A HUGE clean-up operation continued last week in Alaska's Prince William Sound, the site of the largest oil-tanker spill in US history. As the main oil slick moved southwest, out of the sound, it left behind smaller slicks, hundreds of miles of contaminated shoreline and more than 1,400 square miles of fouled waters. The wrecked tanker *Exxon Valdez* was floated from the reef where it had run aground and towed to a nearby island for repairs.

The captain of the tanker, Joseph Hazelwood, is said to have been under the influence of alcohol and absent from the bridge at the time of the accident. Coast Guard investigators were told by at least one crew member that Hazelwood had placed the ship on automatic pilot on an erroneous collision course with the reef.

Hazelwood, wanted in Alaska on misdemeanor charges associated with the spill, surrendered to the authorities in his home state of New York last week. A New York judge set his bail at \$1 million, after pointing out the magnitude of the disaster, but a second judge later reduced bail to \$25,000.

The oil contaminated a large portion of the herring-spawning grounds in Prince William Sound. Last week, Alaska Fish and Game officials cancelled the herring-roe fishery that would normally have opened several weeks ago. Paul Ruesch, a state fisheries biologist, said both herring eggs and young larvae are highly susceptible to toxic hydrocarbons. "We couldn't justify additional mortality from the fishery", he said. There is also a risk that the roe may be contaminated and therefore unsaleable. The fishermen, who stand to lose \$12 million, hope to recover damages from Exxon.

This year's \$70-million salmon fishery, due to begin in early summer, may escape serious damage from the spill, but the catch in future years is likely to be reduced if the oil affects this year's young. Fishermen continued to set floating booms to keep oil out of the bays that house the area's four salmon hatcheries.

But Ruesch acknowledged that success with booms has been "spotty", and the hatcheries are largely at the mercy of the winds. If large amounts of oil come their way, salmon fry reared in captivity may be caught at a vulnerable stage when they are being transferred to salt-water pens ready for their May release. Wild fry which are beginning to emerge from gravel beds will also be at risk.

The level of toxins in the sound, and their effect on the plankton, is not yet known, as water sampling has just begun. Various agencies are preparing for long-term studies of the effects of the oil on water quality, sediments and beaches.

"There will undoubtedly be some very long-term persistent impacts", said Ruesch. "Prudhoe Bay crude does not weather well; it doesn't break up and it doesn't sink. It is very persistent."

The sound has the largest concentration of sea otters in the world, according to Mark Kuwada, a habitat biologist with the fish and game department. Kuwada said that 30 per cent of the sound's 5,000 sea otters live in areas affected by the spill, although it is not known how many have died.

Thousands of waterfowl have been killed by the oil, and the sound's large population of endangered bald eagles has been reported to be feeding on the contaminated carcasses, although no dead eagles have yet been found. Two deer were found dead after apparently feeding on seaweed tainted with oil.

President Bush last week made available US military troops to help clean up the spill, acknowledging that Exxon's efforts were insufficient. He emphasized that the government was not "federalizing" the clean-up operation, and that Exxon would still be responsible for costs.

Environmentalists say an accident such as this was inevitable, given Alaska's laxity in monitoring the oil industry, which supports 85 per cent of the state's economy. Mike Matz, of the Sierra Club, said Alaska's Department of Environmental Conservation, which is responsible for approving the oil industry's contingency plan for clean-up of spills and for assuring that the industry maintains the promised level of preparedness, lacks adequate funds and has been unable to carry out adequate monitoring. Even when permit violations were discovered, said Matz, political pressures prevented prosecution of the violators.

Although the spill has not changed the Bush administration's support for oil exploration in Alaska's Arctic National Wildlife Refuge (ANWR), public outrage over the accident has slowed the progress through Congress of a bill to authorize the exploration, while both houses hold hearings on the causes and potential effects of the accident. Congressional representatives from California have used the spill to fuel their protest against the opening of areas off the California to oil exploration. Interior secretary Manuel Lujan Jr warned oil industry representatives last week that the *Valdez* accident could have an effect on the oil industry similar to that of the Three Mile Island accident on nuclear power. "If the image of an uncaring and uncaring industry prevails among the public", he said, "then we can kiss goodbye to domestic oil and gas development in ANWR, offshore, and in the public lands."

Marcla Barlnaga

FRENCH RESEARCH

Worries about future

Paris

THE French research minister, Hubert Curien, has set up two working parties with unions representing researchers to find ways to protect the future of their profession. One will consider the problem of recruitment of young researchers to the major state research organizations, such as INSERM and CNRS, while the other will look at career prospects, with the accent on greater mobility of trained researchers towards universities and industry.

The unions are pessimistic about the negotiations. Robert Descimon, general secretary of SNCS, the biggest union representing full-time researchers, fears that the government is dismantling the state research organizations in favour of the universities, whose research role in many disciplines is relatively small. Most research is carried out in state organizations, where researchers have jobs for life and a small teaching load. But a slowing in the number of new posts has created a bottleneck of young postdoctoral researchers seeking their first job, with a resulting rise in the average age of researchers. Meanwhile, salaries are becoming increasingly unattractive compared with those offered by industry, even to untrained researchers.

In his pre-election "letter to the French people", President François Mitterrand declared that research would be a national priority. "There is not much evidence of this so far", says Descimon. Most of the government effort, he says, is directed towards major reforms of education, including higher education. The unions fear that trained researchers will be drained from the state research organizations in order to inject new blood into the universities, without the promised reevaluation of research as a career.

Peter Coles

JAPAN

Electronic Leonardo

Tokyo

JAPANESE art lovers who want to see the Mona Lisa but cannot make the trip to the Louvre may soon be able to enjoy the next best thing. A new art gallery in Gifu, central Japan, is using high-definition television (HDTV) to display works of art recorded digitally on compact discs.

HDTV, which was first developed by Japan's National Broadcasting Corporation (NHK), has twice as many lines on the screen as conventional television and produces images of exceptional clarity.

The Gifu Museum of Fine Arts is using the system to show pictures from its collection which it does not have room to put on display. In the future, museum officials hope to import HDTV images from art galleries all over the world so that visitors will be able to view famous works of art at the push of a button.

David Swinbanks

Activists attack in Arizona

Tucson

ANIMAL rights activists broke into four buildings at the University of Arizona on 3 April and set two fires, vandalized laboratories and kidnapped 1,000 animals to protest against the use of animals in research. University officials said that structural damage amounted to \$110,000 and estimates of the total damage go as high as \$250,000.

In press releases left at local newspaper offices and radio and television stations, the Animal Liberation Front claimed responsibility for the arson and damage. A fire in a rooftop laboratory of a five-storey pharmacy-microbiology building gutted two rooms and damaged two others, causing a total of \$90,000 damage. That fire took an hour and 46 firefighters to get under control.

A second fire was started in the crawl space under the small house containing the animal resources administrative offices and caused \$10,000 damage. Clyde S. Card, director of the Arizona Diagnostic Laboratory, said that about \$125,000 damage had been done to that laboratory's autopsy room and three veterinary science laboratories.

Vandals also smashed windows and ransacked laboratories in a neighbouring building and in another a block north of the pharmacy-microbiology building. They spray-painted slogans including "Nowhere is safe", "We shall return", "You are killers" and "Torture teaches nothing" in red on the walls and floors. Pet food was strewn over one basement.

The group's statement said that a thousand animals were liberated as an act of mercy and compassion for the individual animal victims and that laboratories were damaged to protest against vivisection. The statement claimed that the action was part of an international campaign against misguided efforts by the scientific and medical industry.

'People for the Ethical Treatment of Animals', an animal rights organisation based in Washington, DC, later provided television stations with a video of the break-in, showing black-hooded individuals loading animals into boxes and smashing windows with hammers. The group took about 1,000 mice, 50 rats, 175 rabbits, 4 frogs and several guinea pigs. According to the animal activists, the animals were being placed "in good homes around the country where they will live free from the invasive curiosity of researchers and vivisectionists".

About 30 of 340 mice taken from one building were infected with *Cryptosporidium*, a protozoa that causes severe diarrhoea, according to Charles Sterling, a professor of veterinary science.

The mice were being used in a study

supported by the US Environmental Protection Agency to test the effectiveness of disinfectants against this waterborne parasite. Those who handle the mice may suffer from severe diarrhoea for two to four weeks.

Other affected work included genetic studies of heat stress, studies of the long-term effects of selenium-deficient diets, research on a vaccine for swine dysentery and studies of the effect of diet on protein metabolism. Scientists involved estimated that the loss of animals set the projects back months, and perhaps years.

The Animal Liberation Front group may have been helped by people inside the university as they appear to have known where and how the animals were housed, according to Michael Cusanovich, University of Arizona vice-president for research. The group is thought to have broken into one building by forcing open a vent. Police were not sure how the group gained access to the other buildings. The Federal Bureau of Investigation is also involved in the case.

In January, animal activists claimed responsibility for taking seven dogs used in heart research at the Tucson Veterans Administration Hospital.

Elizabeth Pennisi

Municipal law on its way

Boston

WITH recommendations from an advisory panel due this week, the city of Cambridge, Massachusetts, moves one step closer to passing the first local ordinance in the United States to provide municipal control over the care and treatment of laboratory animals. While it is still too early to know precisely what provisions the law will entail, it is likely that the city council will pass legislation this spring.

The new recommendations come from a panel established by the city council in the autumn of 1987 to evaluate the treatment of laboratory animals at facilities in Cambridge. Among those facilities are several belonging to the Massachusetts Institute of Technology (MIT).

The three-member panel, which includes a local veterinarian and representatives from the scientific community and animal rights groups, found no instances of cruelty or abuse of animals in Cambridge laboratories. But the group is still split over the adequacy of existing regulatory mechanisms in the city.

Because of the disagreement, the panel delayed release of its recommendations to the city council. But the pressure is on for city officials to act on the issue. Under consideration are provisions which would give a local animal commission the power to uphold "the community's ethical standards for the use of animals in experiments". The local authority would investigate alleged violations of local standards for the care of laboratory animals and fines to research institutions that failed to comply. A further provision to be considered by the city council would require researchers who handle animals in laboratories in Cambridge to take a course from the local animal commission on the new standards.

John Moses, an MIT physician who heads the university's animal care committee, represented the scientific community on the advisory panel. He stresses

that the panel's lack of evidence of animal abuse argues against the need for local legislation. But he believes the panel is "very close" to making recommendations that all three representatives can agree upon. The recommendations should give a clear guide as to the severity of the city council's actions.

The council has already taken interim action. Nearly two years ago, when the advisory panel was formed, it banned outright within city limits the use of two common animal toxicity tests, the Draize test and the LD 50 test. Animal rights groups have already proposed strong city legislation to regulate practices involving laboratory animals; the panel's forthcoming recommendations are seen as offering the potential of a politically viable compromise.

With the week of 24 April designated by animal rights activists as 'world week for lab animals', increased public attention is expected. In Massachusetts, organizers say they plan to hold demonstrations aimed specifically at the Gillette Corporation because of the company's use of animals in the development and testing of its products.

In New York, for the third year in a row, protesters plan to target their demonstrations at New York University. Animal rights groups have singled out the research conducted by Ronald W. Wood, professor in the department of environmental medicine. Wood, who studies the effects of the inhalation of toxic substances including crack and cocaine, as well as organic solvents, uses rodents and some primates in his research. Last year's demonstrations in New York drew more than a thousand protesters.

Wood says he believes most are "well intentioned, but misguided". "My work", he says, "tries to provide information about very real human problems of drug abuse and occupational hazards."

Seth Shulman

Different ways with *glasnost*

London

Mr Mikhail Gorbachev, the Soviet president and Party general-secretary successfully tied up London traffic for two days last week, made a speech at the Guildhall, had lunch with the British Queen, who accepted his invitation to visit the Soviet Union at a "convenient" time.

The official visit to London followed one to Cuba, both of which had been postponed in December on account of the Armenian earthquake. But the changed itinerary allowed Gorbachev to meet the Prime Minister of the Irish Republic during a stopover on the outward journey.

The three visits showed some interesting contrasts in the exercise of *glasnost*. In Ireland, Gorbachev stressed his concept of a "common European home" in language apparently innocent of Ireland's jealously guarded neutrality, but agreed with Mr Charles Haughey on further economic collaboration in fishing, shipbuilding and barter in food products.

Gorbachev promised to raise in London two human-rights issues arising from the continuing conflict in Ulster (Northern Ireland), but declined to suggest a political solution for the conflict.

In Cuba, where President Fidel Castro has so far proved remarkably resistant to *perestroika*, the emphasis was on continuing friendship. The two leaders signed a new treaty of cooperation in science and

technology, and promised improved contacts in "science, culture, education, health-care, the press, radio, television, cinema, tourism and sport".

But Gorbachev gave a clear warning against the "export of revolution" from Cuba to Latin America and visited a science exhibition showing a new high-powered sugar-cane harvester, a micro-analysis system for blood testing and a model of the Juragua nuclear power station being built with Soviet assistance.

In Britain, Gorbachev used his Guildhall speech to announce that the Soviet Union has ceased the production of weapons-grade plutonium, and that it is about to do the same for weapons-grade uranium. This was described by the US State Department as an "empty gesture" on the grounds that the recycling of existing stocks of fissile material should suffice for many years to come. Mrs Thatcher, the British Prime Minister, rather emphasized her view that nuclear weapons provide a stronger peace-keeping deterrent than conventional forces.

On the human rights front, the Gorbachev visit was marked by the arrival in London of mathematician Georgii Samoilovich, long denied an exit visa on the grounds of his access to classified information 17 years ago. He is to undergo urgent treatment for a leukaemic condition.

Although the Soviet and British governments agreed during the visit on further cooperation in the arts and trade, little was said at the formal level about science and technology. The Soviet offer to find a place on a future space mission for a British astronaut, now made unconditionally, seems to have run into British unwillingness to meet the costs entailed.

Professor Vitali Goldanskii, director of the Institute of General Physics in Moscow, well-known in Britain as the chairman of the Soviet Pugwash committee, was prominent among Mr Gorbachev's advisers during the whole week. As one of the first to recognize that some radioactive nuclei may emit particles heavier than α -particles, he was one of those who visited the Atomic Energy Research Establishment to inspect the still-negative attempts to replicate electrolytic fusion. He was also eager to explain to all enquirers how he proposes, as part of *perestroika*, to split his Moscow institute into several autonomous units, each with a separate director.

Gorbachev himself visited a British factory manufacturing communications equipment supplied to the Soviet Union — and *Izvestia* took the occasion to lecture the West on the iniquities of the Cocom ban on the export of sensitive technology to the Socialist bloc.

Vera Rich

Remote-sensing satellite for Iraq

São Paulo

Brazil's Institute of Space Research (INPE) is considering a proposal to build a remote-sensing satellite with military potential for Iraq. Brazil was an important supplier of arms to Iraq during the Iran-Iraq war.

INPE, together with Brazil's aircraft manufacturer, Embraer, and private companies would build the body of the satellite using technology INPE has acquired for its own programme to construct four satellites. Brazil will have its first satellite ready by the end of the year, although it may have to wait until 1992 for an indigenous launch. The development of the Satellite Launch Vehicle by the Air Force is behind schedule.

The first two Brazilian satellites will be small (115 kg) test capsules. Remote-sensing satellites of similar size will then be launched. INPE also has an agreement with China to design a bigger remote-sensing device.

Iraq is thought to be interested in help in establishing a satellite-building and testing facility. The sophisticated camera required for military sensing would come from a French company. But the deal appears to be encountering opposition inside Brazil's Foreign Ministry. INPE's director-general, Marcio Nogueira Barbosa, said he would prefer to pass INPE's satellite-construction technology to private companies.

Ricardo Bonalume Neto

Study planned of greenhouse effect

Sydney

A YEAR after rejecting a request for A\$1 million to support research into the greenhouse effect, the Australian government has changed its mind and agreed to spend A\$7.8 million on the same topic over the next 15 months.

The Prime Minister, Bob Hawke, explained the turn-around by stressing the "dramatic explosion of awareness" of the greenhouse effect over the last year.

Part of the A\$7.8 million will be used to set up a National Greenhouse Advisory Committee of six experts to help the government to set research priorities. A\$5.54 million will go to the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Bureau of Meteorology to continue research into predictive modelling. The remainder will support Australian participation in international research programmes.

The government has also promised to help countries in the Pacific region that will be affected by changes in sea level.

Tania Ewing

NEWS IN BRIEF

New radiotelescope

Bangalore

Under a new agreement, India and the Soviet Union will jointly fabricate and install a radiotelescope in South India. The telescope will form part of an eastern hemisphere very-long-baseline interferometer. It will be linked to the baseline connecting similar radiotelescopes at Ussurisk and Ephratoria in the Soviet Union.

R. R.

Prisoners freed

Washington

TWELVE Somali scientists, engineers and medical doctors were freed from prison earlier this month, according to two committees of the US National Academy of Sciences and the Institute of Medicine which had taken up their cause. A report published by the two committees last year accused the Somali government of torturing prisoners and of detaining them for years without formal charges (see *Nature* 331, 196; 1988). Somali Prime Minister Lt General Mohamed Ali Samantar promised in a speech in Washington last month that his government would release the prisoners.

J. P.

Having the final word. . .

SIR—I do not know whether there are still Creationists who believe that God created the world with implanted fossils and strata records (and similarly a Universe with implanted galactic records); but, if there are, then Bruce Denness's letter "Divine artefact" (*Nature* 336, 614; 1988), raises a problem that should be of great concern to them.

"God is not a man, that He should lie" (Numbers 23:19). Yet the suggestion that God could have created a world, or Universe, in which a record of the past was 'implanted', implies that he has lied. And the consequences to faith could be more shattering than they are to science.

First: if (*arguendo*) the Earth and Universe are (say) only 20,000-odd years old, but have been created to appear respectively about 5×10^9 and 20×10^9 years old, then these greater ages are without doubt their *scientific* ages — there is no way that science can come up with an age of 20,000 years. To understand this, consider what the *scientific* ages of brand-new but exact copies of this Earth and Universe would be, if God chose to make such copies.

Second, it becomes a matter of faith alone, under such circumstances, that the age of the Earth or Universe is anything other than what it appears to be scientifically. And only faith can govern the selected age. But why, under such circumstances, should any particular age be arbitrarily chosen? Creationists may interpret the Bible as implying one age rather than another; but the Earth could have been created *some year ad* with Bibles intact as part of the implanted record. How is one to prove that that did not occur? But if it did, then Jesus was not born on Earth and did not die for us; nor was he resurrected. What happens, then, to our salvation? So, to believe that God could have created an Earth or Universe with an implanted record ultimately strikes at the heart of the Christian faith. Maybe quite a headache for Creationists.

Perhaps there is no scientific answer to the problem Denness raises, but only a theological one.

JOHN BLASDALE

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SIR—I find that it has become necessary, for a second time (see *Nature* 323, 754; 1986), for Me to intervene in order to present My Word in these pages.

Unless I have misunderstood matters (which is unlikely), I am depicted by some individuals as a sort of Divine Con-Artist (*Nature* 336, 614; 1988 and 337, 498; 1989), allegedly creating the Universe, and then disguising this fact, for reasons apparently known only to Myself. Others find it beholden upon themselves to rush

to My defence and deny this, because they have decided that it reflects poorly on My Divine Logic.

I had hoped that My previous Correspondence in these columns had made clear that *all* speculation of *any* kind on My Nature is unwelcome, from both an epistemological and a Personal view. In particular, if ever again I read Professor Einstein's remarks to the effect that, whilst I am Cunning, I am not Malevolent, the extent of My lack of malevolence will be sorely tested. It is not that I disapprove of Professor Einstein's flattering description, it is that even My Patience, Infinite though It is known to be, is beginning to crack at the many, many repetitions I see of that comment. One other observation I hope never to be quoted again, at least for a long time, is Professor Haldane's, the one about the cosmos being not only stranger than one imagines but . . . you know how it goes. Whilst apposite, I feel it needs a rest.

But I digress (which is one of the qualities One finds in Omnipotence).

A periodical ostensibly concerned with matters of scientific interest would do well to think carefully before allowing speculations of a totally metaphysical nature space in which to appear. I certainly would hope that I am as disposed to be as Broad-minded as the next Entity and that no-one would accuse Me of desiring to fetter a free press, particularly in this country at this time, but I commend the matter to your most earnest consideration, if you get My drift.

I trust I shall not have to bring this to your attention on a third occasion.

GOD

(As revealed to Ralph Estling)

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■ It is to be hoped that putative correspondents will take the hint. Editor, *Nature*.

Intuitive science

SIR—I have been reading *The Value of Science* by Henri Poincaré, who believed that intuition and logic make science advance. Intuitive scientists work in figures and imagination; in mathematics, they are its geometers. Logic scientists go from the particular to the general; they are the analysts in mathematics.

As a biochemist who agrees with Poincaré, I ask: what is the contribution of logicians and analysts to the advance of biochemistry? From the biochemical journals, it seems that only logicians exist. All hypotheses, even if they are subsequently shown to be wrong, are based on experi-

mental results presented in a formal and statistically rigorous way.

I believe that intuitive biochemists exist, but that their contributions have to be communicated in the form required by the journals, in analytic form. Hypotheses that are intuitive in nature are not considered suitable for biochemical journals.

Yet Poincaré points out that intuitive work is even more important than analytical work for the advance of science. In biochemistry, it sometimes seems that analytical work is performed without any precise objective. The thousands of articles in the journals contribute very little to the advance of knowledge. That is why two urgent needs in biochemistry are (1) the study of all the available information in order to advance intuitive hypotheses and (2) the recognition and promotion of intuitive biochemists through publication of that work. To these ends, journals should change their policies without lowering their quality.

Poincaré said it clearly: "Pure logic does not lead to anything but tautologies; it creates nothing new".

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History lesson

SIR—You remark in your unsigned Commentary article marking the fiftieth anniversary of the discovery of uranium fission¹ that to suppose that "some history is so painful that nothing can be learned from it . . . is a disservice to the intellect" bears on three items in the previous issue of your journal²⁻⁴ dealing with quark matter and strange matter. In the News and Views piece on the subject², we find the statement that the growth phase of quark nuggets caused by the addition of low-energy neutrons "releases of the order of 20 megaelectron volts of energy yield per captured neutron . . . nearly 10 times the yield per neutron in a conventional fission reactor".

Let us hope that the human intellect has matured enough in the intervening 50 years to learn from past experience, and that if the proposal of Shaw *et al.*³ for quark matter engineering turns out to be successful, you will be able to write a different kind of Commentary for *Nature* in 2039.

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1. *Nature* 337, 499–502 (1989).

2. Alcock, C. *Nature* 337, 405 (1989).

3. Shaw, G.L., Shin, M., Dalitz, R.H. & Desai, M. *Nature* 337, 436–439 (1989).

4. Brügger, M. *et al.* *Nature* 337, 434–436 (1989).

Hot-footed towards cold fusion

The only published account so far of thermonuclear fusion in an electrochemical cell raises, as its authors say, "more questions than it provides answers".

THERMONUCLEAR fusion, which allows stars to shine, must also occur in more humdrum circumstances. Put two deuterons together in a deuterium molecule, for example, and there is a small chance that they will fuse together spontaneously, either to produce a tritium nucleus and a proton or a helium-3 nucleus and a neutron. Perhaps luckily, the chance is astronomically small, maybe 10^{-70} per molecule per second. At that rate, there would be roughly one fusion event an hour in a quantity of molecular deuterium roughly as massive as the Galaxy.

The reason why muons have been advocated as catalysts of fusion is that their mass (some 200 times greater than that of an electron) makes for a smaller D_2^- ion, thereby increasing the wave function of one deuteron at the position of the other and so increasing the still-random chance of fusion.

The great excitement in the past three weeks about the prospect that thermonuclear fusion has been accomplished electrochemically is an extension of this principle. The article by M. Fleischmann and S. Pons which appeared last week (*J. Electroanal. Chem.* **261**, 301; 1989) begins from the view that conditions favourable for fusion may be created electrochemically by exploiting the familiar affinity of palladium for hydrogen (in all its isotopic forms).

As described, the experiments seem straightforward. Use a platinum anode, a palladium cathode and an electrolyte which is a heavy-water solution of deuterated lithium hydroxide. To prevent the deuterons bubbling away as deuterium, arrange that there is a substantial negative over-voltage on the cathode. The effect is that deuterons accumulate in the palladium lattice, and will continue to do so until their conversion into molecules resumes.

That point, Fleischman and Pons argue, is determined by the chemical potential of the deuterons in the palladium lattice, which is itself determined by the negative over-potential on the cathode. They use the term "galvanostatic compression" to describe this process of forcing deuterons into the palladium.

Three kinds of measurements are described, one of which is simple calorimetry. The question is whether the heat produced in such a cell is greater than expected, which is most simply that calculated

by multiplying together the current and the voltage.

The article describes measurements with cells containing electrodes of different shapes and sizes. For three-rod electrodes of different diameters, the specific rate of excess heat production is reported to have increased with increasing current density and with the thickness of the rod (4 mm at its maximum), both of which argue for a phenomenon liberating excess heat in which the bulk of the palladium is involved. There is also an account of how, in one experiment, the cathode (a 1 cm cube of palladium) vaporized.

True fusion should of course be recognizable in other ways, by the detection of its by-product particles for example. The authors report measurements of γ -rays presumed to have occurred by the reaction of neutrons from fusion reactions with protons in the water-bath surrounding the electrolytic cell and also the detection of neutrons (at roughly three times the intensity of the cosmic-ray background) while one experiment was running. They have also looked for (and claim to have found) tritium in the residual electrolyte in a cell.

So does this not add up to a proof of fusion? From the details published so far, no-one can say. The nuclear evidences offered are all close to the edge of what is measurable. The measurement of tritium production, for example, looks convincing, but there are pitfalls. Any quantity of deuterium-rich water invariably contains the heavier isotope in some quantity. One is looking for a small increase in tritium content, rather than its mere presence where there was none before. Similarly, there are other sources of neutrons and gamma rays besides fusion reactions (notably cosmic rays and natural radioactivity), and these must be convincingly subtracted.

Even taking the γ -ray and neutron production at face value, far too few of either are recorded to explain the claimed heat generation by known fusion reactions. In one of the experiments described, the rate of production of excess heat suggests that there should be between 10^{11} and 10^{14} fusion events a second, but the nuclear physics data suggest that known fusion reactions (leading either to tritium or ^3He) account for only about 10^4 of these.

This is the most serious impediment to

belief. The heat production in these electrolytic cells is more than a million times greater than nuclear by-products would suggest it can be. This leads Fleischmann and Pons to say that "the bulk of the energy release is due to a hitherto unknown nuclear process or processes". What, one wonders, can that process be? Is it likely to have escaped the attention of nuclear physicists in the past half century?

Sceptics, in the circumstances, will be quick to ask whether the necessary subtle subtractions from the gross energy output have been accurately made — the electrical energy put in, the heat that would have been produced in a normal electrolytic cell (with ordinary hydrogen instead of deuterium) and so on. Because Fleischmann and Pons spend days, perhaps weeks, loading their electrodes with deuterium, there is plenty of energy stored up in their system before any return is obtained. Even the much-described "explosion" of one of their electrodes may be explicable by chemistry.

By now, the tiny band of *Nature* referees which has scrutinized the two articles submitted for publication has been joined by many other people. Further information about the issues which have given them difficulty will be given in a further issue of *Nature*. Numerous attempts to replicate the measurements have produced only one positive claim (see page 529). One common complaint has been that the data provided are insufficient to allow faithful reproduction of the measurements reported. The Fleischman and Pons article as now published is a starting-point for other experiments, but says less than the average chemist needs to know.

A more perplexing circumstance is that the authors are reported to have said that some cells work and others do not, although that may not be surprising when so little is known of the system as a whole. The idea that cells produce excess heat only after they have been running for long periods makes sense if a palladium lattice must be charged with deuterons before fusion occurs at anything like a decent rate, but one is left wondering whether the amount of charging needed can be predicted in advance for a given cell geometry and voltage, as one would expect if such a straightforward explanation were the only one. What seems plain is that arguments like these will continue for a long time. □

Where is the Great Attractor?

N. Kaiser

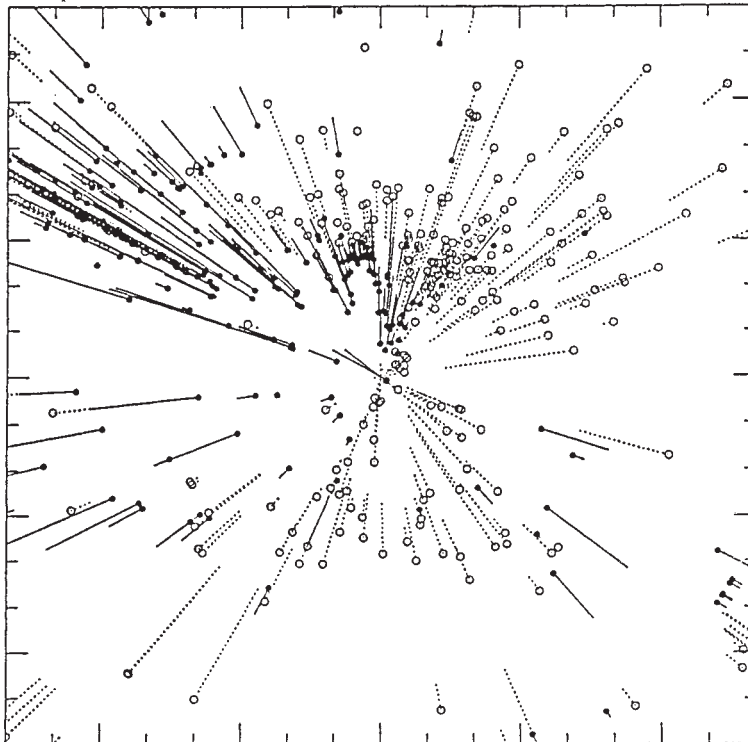
SIGNIFICANT deviations from pure Hubble expansion of the Universe have been revealed by studies of the motion of elliptical galaxies. These have been attributed to the gravitational influence of the 'Great Attractor' — an extended mass distribution whose centre lies at a distance of about $40 \text{ Mpc } h^{-1}$ (the nearest galaxy, for comparison, is about $\frac{1}{2} \text{ Mpc } h^{-1}$ away). The precise scale of this mass excess is the subject of intense debate, with some studies preferring a somewhat smaller distance. Two groups, however, Scaramella *et al.* on page 562 of this issue¹ and Lahav *et al.*², have found a strong concentration of rich clusters of galaxies in the direction of the Great Attractor, but at the much greater distance of about $140 \text{ Mpc } h^{-1}$. Although the gravitational pull of these objects themselves is probably too small to account for the motions, they may be indicative of an extension of the 'supergalaxy' to a much larger scale than previously thought, with important consequences for theories of structure formation. Alternatively, the alignment may just be a chance coincidence.

The existence of a Great Attractor was initially inferred from studies of the motion of elliptical galaxies³. These revealed that galaxies in the Hydra-Centaurus region, which lie near the apex of the motion of the Virgo supercluster, and so had been suspected as the cause of this motion, were themselves moving in the same direction. This could be explained by the existence of a still more distant mass concentration, dubbed the Great Attractor. Spiral galaxies also show a significant motion in this region of space⁴: the line-of-sight motions for a compilation of spirals and ellipticals (D. Burstein, preprint) are shown in the figure.

Much effort has been devoted to discovering the Great Attractor directly as an excess in the space density of galaxies. One approach is to use redshift surveys to map out the three-dimensional distribution of galaxies and thus infer the acceleration acting on the Local Group of galaxies. These include an IRAS (Infrared Astronomical Satellite) redshift survey^{5,6} and Dressler's survey⁷ which concentrates on the Great Attractor region. An

alternative is to use the much larger catalogues which have apparent luminosities but no redshift information — the idea here is that as both light and gravity obey inverse-square laws, the dipole moment of the extragalactic light should be proportional to our acceleration and thus to our motion, provided that the surveys extend far enough to encompass the source of our acceleration.

These studies seem to confirm the



Positions and line-of-sight peculiar velocities of local galaxies: box, centred on our Galaxy, is of side $80 \text{ Mpc } h^{-1}$. Hydra Centaurus lies in the upper-left quadrant, and the Great Attractor, as seen from the trend of the motions, is beyond the left edge. (Broken vectors, motion towards us; solid, away.)

existence of a significant excess of galaxies at about the right scale and direction. It is particularly impressive that the dipole moment of the extragalactic light agrees in direction with our motion to about $10\text{--}20^\circ$, and although there are differences — the IRAS redshift survey suggests a somewhat smaller scale for the attractor — the general impression is of a fairly consistent picture in which we can understand at least the gross features of the velocity field as the response to the gravitational

attraction of matter.

The theoretical implications of all this are less clear. A leading problem in cosmology is how the physics of the early Universe influenced the structure observed now (see also page 541). In particular, it is thought that fluctuations in the density of (perhaps unobservable) matter could have initiated the formation of galaxies and be responsible for the observed non-uniform distribution of the galaxies.

It seems, on the one hand, that the scale and amplitude of the mass profile implied by the Great Attractor are uncomfortable for the most popular cold-dark-matter model^{8,9} (and this has stimulated interest

in alternative theories with extra large-scale power), but this is based on a rather literal interpretation of the model. The problem is that the Great Attractor model, which accounts quite well for the data, also makes predictions about the velocity field in regions where there is little or no data — on the far side of the Great Attractor for instance — so it is difficult to interpret this apparent discrepancy. On the other hand, the amplitude of the extragalactic light dipole moment agrees well with the predictions of cold dark matter.

A more recent development has been the application of correlation analysis^{10,11}. The authors have tried to provide a measure of the scale and degree of coherence of the motions with a method similar to that used by Peeble in a powerful analysis of galaxy cluster-

ing. The conclusions here seem at first sight to be in conflict, but this is probably a consequence of the different weighting schemes used which mean that the statistics sample the velocity field at quite different distances. The less myopic sample¹⁰ gives results which are not greatly out of line with the theoretical predictions. What is apparent from all these studies is that we do not yet have a sufficiently large survey to constitute a 'fair sample'.

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Although the viability of the cold-dark-matter model, in the face of the velocities shown in the figure, remains very much an open question, the structures reported by Scaramella *et al.* in this issue¹, which are also apparent in X-ray cluster catalogues² threaten a much wider range of theories. The major concentration of these rich clusters is so distant that it should not have contributed significantly to the acceleration inferred from either the redshift surveys or from the extragalactic dipole moment. In theories for the formation of structure which invoke gaussian random fields for the initial fluctuations that stimulated galaxy formation, one would not expect the mass excess at such distances to be aligned with the nearer mass excess, so even in theories with excess large-scale power the alignment seen is something of an embarrassment.

LYMPHOCYTE DIFFERENTIATION

Not all in a name

Stephen Shaw

THE ambition of participants in the international workshops on leukocyte differentiation antigens has become nothing less than the definition and functional characterization of the entire array of cell surface molecules on human leukocytes^{1,2}, which by the end of this year's meeting* stood at a total of more than 78. These molecules now provide a fertile common ground, uniting the interests of immunologists, cell biologists, biochemists and those in other disciplines.

About half of the 33 newly classified molecules were previously unknown or quite obscure. For such molecules, the standardization of nomenclature and definition of reagents provides a starting point for investigation and collaboration. For some of them, the synthesis of information that occurred at the meeting was dramatic; arguably the most exciting was for a molecule now designated CD59. Based on functional, biochemical and molecular-biological approaches using two monoclonal antibodies (V. Horesji, Czechoslovak Academy of Science, Prague; G. Hale, University of Cambridge), the phosphoinositide-linked CD59 molecule is now thought to be the human homologue of murine Ly6c, a molecule capable of transmitting a T-cell activation signal³. Less direct evidence suggests that it is also identical to the molecule H19 (N4) which participates in T-cell adhesion to erythrocytes⁴.

The rest of the 'new' molecules were already reasonably well defined before the meeting. For some, such as LFA-3

These observations are not the first to suggest that the local supercluster is only part of a much larger structure¹², and they lend support to this view. Whether this spells the end for conventional theories remains to be seen; perhaps the alignment is an acceptable chance occurrence; the X-ray cluster analysis involves a rather small number of objects, and the reliability of the Abell catalogue for these studies has recently been called into question¹³. Further studies are needed to establish the real extent of the supergalaxy, but have no doubt that in the meantime theorists will rise to the challenge and produce new theories to account for this tantalizing observation. □

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achieved by the workshop enriches our understanding of molecules by fostering cross-cultural exchanges of information regarding them.

In previous workshops of this series, the emphasis was largely in characterizing molecules whose expression is restricted to specific lineages of lymphoid cells such as CD3 (T3), CD19 (B4) or CD13 (MY7). In the present workshop, the emphasis shifted to molecules expressed on multiple cell types within and outside the haematopoietic lineages — not only because there are many more such molecules to define, but also because their functional importance is beginning to be appreciated. The interplay of lineage-specific molecules with molecules of broader distribution is evident in T-cell activation. The great specificity of T cells for particular foreign antigens is conferred by the T-cell receptor, a highly variable immunoglobulin-like heterodimer which associates with many other polypeptide chains to form the CD3 complex. Crosslinking of this receptor complex initiates intracellular signals including hydrolysis of phosphoinositol, Ca²⁺ mobilization and protein kinase C activation.

An additional invariant T-cell-specific receptor, CD2 (T11), whose physiological function remains to be determined, activates a similar cascade of signals when perturbed by particular pairs of monoclonal antibodies. CD2 or CD3 generally fails fully to activate T cells by themselves; additional signals generated by other cell-surface molecules are necessary to initiate T-cell activation or to augment it. More than 15 surface molecules have been inferred to play such a role in altering T-cell activation; although many such molecules are T-cell specific (such as CD5, CD28), many others are not (such as CD43, CD45), including four newly implicated at this workshop (see table). Systematic approaches such as that described by S. Huet (Inst. Gustave-Roussy, Villejuif) promises to bring some order out of the chaos; she finds consistent patterns of complementation between particular pairs of incomplete signals mediated by CD2, CD3, CD5, CD27 and CD28.

Does this level of complexity reflect T-cell physiology, or elaborate artefact? Although the question remains open for

(CD58), ICAM-1 (CD54), and the transferrin receptor (CD71), it was a useful but unglamorous achievement to assign a standard nomenclature and to validate reference reagents. For others, such as the neural cell adhesion molecule N-CAM, ecto 5'-nucleotidase, decay-accelerating factor (DAF) and various molecules of the integrin family, it served notice that molecules being 'discovered' in one context are well known to those in other disciplines.

First, for example, CD29, used by T-cell immunologists as a marker of T-cell differentiation, is now understood to be the β -chain of the VLA family of integrin adhesion molecules. Second, CD56, known to immunologists as NKH1, a marker of natural killer cells, turns out to be an isoform of N-CAM. Third, many of the molecules are now being appreciated to be enzymes, including several different classes of peptidases: CD10 (ref. 5), CD13 (T. Look, St Jude Children's Research Hospital, Memphis) and CD26 (T. Mattern, Forschungs-Institut Borstel); ecto 5'-nucleotidase CD73 (L. Thompson, Scripps Clinic and Research Foundation, La Jolla); and the complement regulatory DAF protein (CD55). Thus, the unification

Molecules not restricted to T cells which are newly reported to initiate or augment T-cell activation.

CD	Other names	Reporting laboratories
CD23	B6	L. Goff, ICRF Tumour Immunology Group, London
CD54	ICAM-1	C. Cerdan, Unite 119 INSERM, Marseille
CD44	Hermes, Pgp-1, ECMRIII	S. Denning, Duke Univ., Chapel Hill S. Huet, Inst. Gustave-Roussy, Villejuif Y. Shimizu, NIH, Bethesda
CD73	ecto 5'-nucleotidase	L. Thompson, Scripps Clinic, La Jolla

*Fourth international workshop: *Leukocyte Differentiation Antigens* Vienna, 21–25 February 1989. Organization of the next workshop is by R. Winchester and T. Springer. Summary table for antigen classification will appear in *Immunology Today* in August.

many of the molecules, biochemical mechanisms are being defined to account for regulatory effects of some of the best-studied molecules. CD5 mediates an increase in PIP2 precursor pool for phospholipase C (ref. 6), and CD28 stabilizes specific mRNA transcripts⁷. The 'non-lineage' CD45 molecule, whose isoforms are proving to be extraordinary markers of lymphoid differentiation⁸, has recently been demonstrated to have an intracytoplasmic region which is a tyrosine phosphatase⁹. B. Schraven (Deutsches Krebsforschungszentrum, Heidelberg) reported that CD45 monoclonal antibody augments T-cell activation principally when activation occurs via the CD2 molecule, and that CD45 is physically associated with CD2, based on crosslinking studies. This finding, if confirmed, lends credibility to the model proposed by Ledbetter *et al.*¹⁰ that CD45 regulates activation by association with and potentially by dephosphorylation of other receptor molecules. Together with the findings that CD4 and CD8 associate with p56 *lck* tyrosine kinase^{11,12} (C.E. Rudd, Sidney Farber

Cancer Institute, Boston), there is potential for the complex regulation of T-cell activation by tyrosine phosphorylation and dephosphorylation. □

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SEMICONDUCTOR CLUSTERS

Getting smaller by design

Michael Grätzel

SUBMICROSCOPIC clusters of semiconductor material are remarkable for the unusual properties they acquire through the quantum effects of their small scale. Two groups now report unusual synthetic routes by which cadmium sulphide clusters of specific sizes can be grown. Herron *et al.*¹ use zeolites, porous crystalline structures, as hosts to networks of CdS clusters which they term superclusters and which have additional quantum properties. And on page 596 of this issue², Dameron *et al.* describe yeast cells that synthesize CdS crystallites as a means to

prevent cadmium poisoning in cadmium-bearing solutions.

Colloidal semiconductor particles 1–100 nm across are a new class of materials whose properties are intermediate between those of isolated molecules and solids. Apart from their potential application in the area of photocatalysis and solar-energy conversion³, they offer a convenient vehicle for studying the transition from the molecular to the bulk state. For example, several recent studies on semiconductor colloids have shown that their optical properties depend on their size —

the so-called quantum-size effect — and are significantly different from those of the macroscopic crystals.

Size quantization effects have been widely studied in the past for metal particles. In bulk metals, considered as the limit of an infinitely large system, there is a continuous spectrum of electronic states. In contrast, in very small particles, the energy levels are quantized, the spacing between adjacent states being of the order of E_F/N , where E_F is the Fermi energy — the characteristic energy of the most significant electrons in the system — and N is the number of atoms or

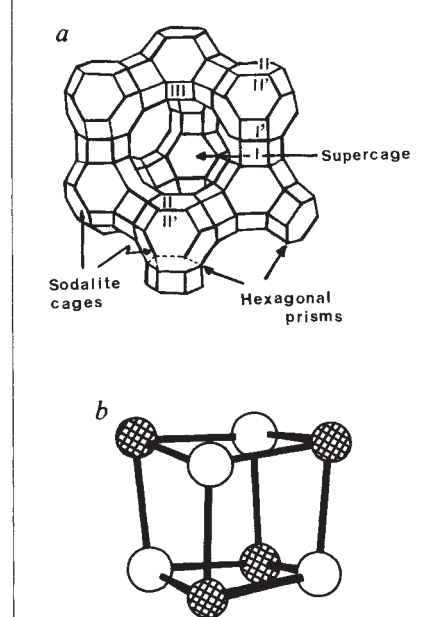


FIG. 2 a, Structure of zeolite Y and X, used in the study of Herron *et al.* b, Structure of the tetramer CdS(O) clusters located¹ within the zeolite/sodalite unit; (solid circles Cd, open circles S(O)).

molecules in one particle. Because E_F has a value of a few electron volts, the step size in a particle containing, say, a thousand atoms, is several millielectron volts. This quantization of states produces several anomalies in the properties of the particles, including their optical absorption, as was recognized 50 years ago.

For semiconductors, much of the earlier effort has concentrated on two-dimensional structures, such as quantum wells and superlattices. In thin films of semiconductors, a size quantization perpendicular to the film occurs. Three-dimensional systems displaying confinement effects, sometimes referred to as quantum dots, are the focus of more recent interest. Quantum-size effects in such semiconductor clusters were first observed by Berry⁴ in studies of silver halide crystals.

As the particle size is decreased, the band-edge absorption feature in the material's spectrum, corresponding to the minimum excitation, moves to shorter wavelengths (higher energies), a blueshift which is attributed to the local confinement of the electron-hole pairs (Fig. 1). This produces discrete electronic states in the valence and conduction band of the colloidal semiconductor and increases its effective band gap (E_g). The blueshift in the absorption threshold is a consequence of the increased gap between the highest-occupied and lowest-unoccupied states. At the same time the oscillator strength of the optical transition is reduced and discrete absorption bands can appear in the spectrum. To a first approximation, the energy of the quantized levels is inversely proportional to the square of the particle diameter, because of the quantum effect

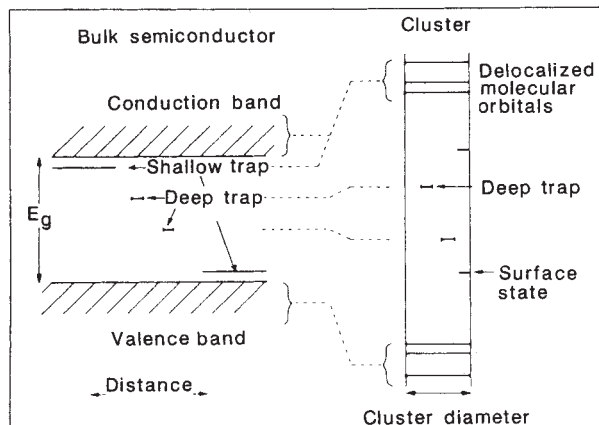


FIG. 1 Spatial electronic-state correlation diagram for bulk semiconductors and clusters. The conduction and valence bands (continuum states) of the bulk system become discrete levels because of the quantum-size effect in the cluster. Shallow and deep traps are associated with defects (impurities and lattice defects) in the crystal.

of confinement, with a correction to allow for the Coulomb interaction⁵ between the electrons and holes. The luminescence of colloidal semiconductors is also strongly affected by particle size⁶. Typically, a broad emission is observed which blue-shifts with decreasing radius of the aggregate.

The synthesis of colloidal semiconductors is often carried out in solution by chemical precipitation. Because larger aggregates are thermodynamically more stable than smaller ones, it is necessary to arrest the growth process at a stage where the particles are still in the quantum-size domain. This is usually done by adding a protective agent, such as hexameta-phosphate, to the solution. However, this method gives a rather broad distribution of particle size, and tedious separation steps are required in order to obtain clusters with fairly uniform morphology.

Herron *et al.*¹ use an ion exchange procedure to incorporate Cd²⁺ into the zeolite subsequently exposed to hydrogen sulphide gas to form the CdS microcrystals. An important finding from their detailed X-ray analysis concerns the location of the CdS clusters. At low concentrations, the Cd²⁺ ions are preferentially adsorbed at the I' site of the zeolite Y (Fig. 2a), so that the clusters are located in the smaller sodalite cages and not, as one might expect, the supercages. The overall structure of the sodalite-cage-entrapped species is a distorted cube containing the tetramer (CdS₄O)₄ (Fig. 2b). The unusual stability of these extremely small clusters is due to the coordination of the Cd²⁺ ions with oxygen of the zeolite framework. These isolated CdS clusters are distinguished by their unique optical properties which deviate greatly from those of bulk CdS. Thus, the absorption threshold in their spectrum is around 350 nm, compared to 540 nm for bulk CdS. The absence of any luminescence emission, even at temperatures down to 4 K, is also conspicuous as large CdS particles show intense emission under similar conditions.

These optical properties undergo drastic changes at higher CdS loadings for which adjacent sodalite cages become occupied by the clusters. The absorption threshold shifts to 420 nm and an exciton-like feature (bound electron-hole 'atom') evolves at 350 nm. Strong emission appears owing to charge-carrier recombination on vacancies and lattice imperfections. These phenomena are ascribed to the formation of CdS 'superclusters' by a percolative process involving aggregation of the tetramers through the double six-rings linking the sodalite moieties. The novel feature of the superclusters is that every CdS molecule in the aggregate is on the surface and that strong coupling with the host lattice occurs through the interaction between Cd and the framework oxygen. This explains the unique optical

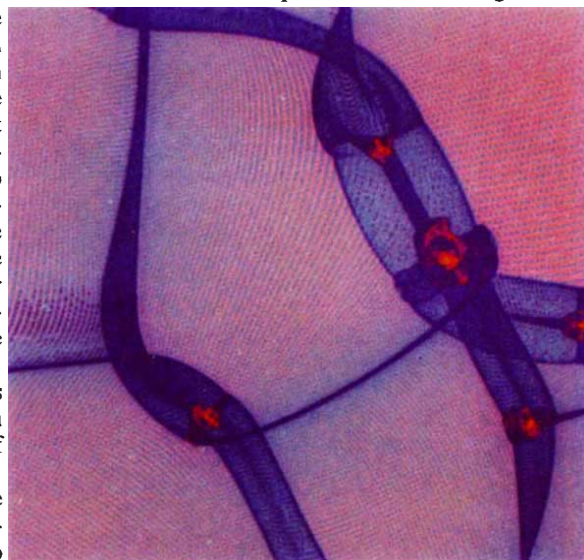
Knotty problems in cosmology

NUMERICAL studies of the gravitational interaction of collections of point masses have been an important tool in 'experimental' cosmology for some 20 years — such interactions underlie the structure of the Universe at all scales. But many computational difficulties remain. To simulate a respectable fraction of the Universe with a limited number of particles, each particle must represent the mass of many galaxies, but now that observational cosmologists are beginning to map complete galaxy distributions throughout large regions of the Universe, the simulators can keep up their side of the work only by following their models at the level of individual galaxies. This means using enormous numbers of particles, which demands huge allotments of computing time.

A common compromise has been to put in large particle numbers, but then to approximate the behaviour of dense regions, as they form, with some kind of fluid mechanical treatment. But S. F. Shandarin (Institute of Physical Problems, Moscow) and A. Melott, working together at the University of Kansas, have returned to the simple but accurate device of calculating individual trajectories for each of 512 × 512 particles on a two-dimensional grid of comparable spatial resolution. Their technique demands too much computing power to achieve useful results in three dimensions, but reveals details which, were fewer particles used, would be invisible.

The scale of the simulations is arbitrary,

but features apparent in the figure resemble structures observed at the scale of superclusters of galaxies. The figure, a detail from one simulation, is colour coded (from red to violet) for the logarithm of the density. The darker regions could correspond to filaments of galaxies



and the intervening patches, 'voids'.

The large structures generally agree with those predicted in catastrophe theory and Zel'dovich's 'pancake' model, but the small-scale structure (such as the voids inside the filaments) is new. Of immediate interest is the suggestion that the small and large structures can be aligned over widely differing length scales. No detailed survey has yet found a scale beyond which the distribution settles to some uniform average. On all dimensions, something seems to be going on, and direct simulations such as these are needed to investigate the controlling influences. David Lindley

properties of these composites. It is noteworthy that CdS clusters in zeolite are distinguished from conventional colloids also by their selective intervention in photocatalytic processes⁷.

Dameron *et al.* describe in this issue² the biosynthesis of quantum-sized CdS crystallites in the yeast cells *Candida glabrata* and *Schizo saccharomyces pombe*. When exposed to cadmium ions, these cells respond with the synthesis of γ -glutamyl peptides, (γ -Glu-Cys)_n-Gly and an enhancement of sulphide production. As a result, small crystallites of CdS are produced inside the cells. Surprisingly, the colloids crystallize in the rock-salt structure instead of the hexagonal configuration which is the thermodynamically stable form of CdS. The role of the peptide is that of a protective agent: the cysteinyl thiolates in the peptides are surface ligands to the core of around 85 CdS units. In this way, the organism controls particle

nucleation and growth yielding uniformly sized CdS particles of approximately 20 Å diameter. Not unexpectedly, the optical absorption of these aggregates reveals pronounced quantum-size effects as in the case of the zeolite contained CdS clusters. The findings of Dameron *et al.* provide a first example for the biosynthesis of quantum-sized semiconductor crystallites constituting a unique metabolic route for the detoxification of Cd²⁺-infected living cells. □

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Pathogenesis by antisense

Bob Symons

THE first determination of a nucleotide sequence of a 7S RNA species in plants is reported in a recent issue of the *EMBO Journal* by Heinz Sanger and colleagues¹. This 7S RNA, isolated from tomato leaf tissue, is very similar to the known sequence of 7SL RNA from mammals, which prompts Sanger and colleagues to suggest that the two RNAs, both 299 nucleotides long, have similar functions.

Mammalian 7SL RNA, in combination with six proteins, forms the signal-recognition particle (SRP) responsible for protein translocation after synthesis². In their new work, Haas, Sanger and colleagues produce a computer-generated model of the secondary structure of the tomato 7S RNA and report that its two-dimensional structure is also very similar to that of the mammalian species.

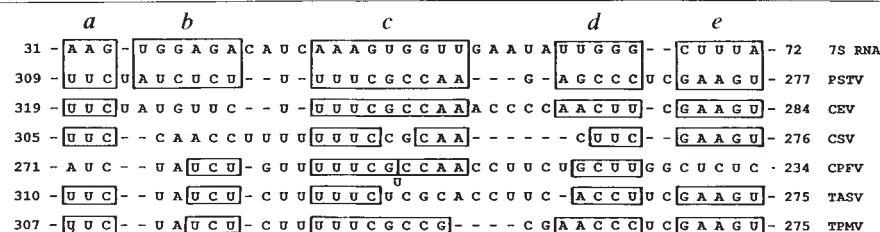
The more intriguing and unexpected aspect of the sequence and structural analysis of the tomato 7S RNA is the suggestion¹ that the pathogenic effects which viroids can cause in plants on infection are a consequence of the formation of a complementary hybrid between the viroid and 7S RNA. Viroids are the smallest infectious agents known³, consisting of circular, single-stranded RNA molecules which vary in length from 246 to 375 nucleotides. About 15 have been described so far, and most are pathogens that significantly affect agriculture. Many have a wide host range, infecting higher plant species, whereas others are restricted to a single family. Symptoms of infection vary

widely but the most common are dwarfing of the whole plant, curling downwards of the leaves (epinasty), and the appearance of yellow blotches on the leaves in certain hosts.

There is hardly any information as to how these intriguing agents exert their effects. One important aspect is the comparatively long time it takes for viroids to

plementary sequences in the tomato 7S RNA (see figure). The pathogenic effects of PSTV could be mediated by the formation of a 7S RNA–PSTV hybrid *in vivo* that would interfere with the normal role of 7S RNA in protein translocation across membranes. As the authors point out, viroid infection can lead to abnormal formation of cellular membranes.

Such hybrid formation would presumably need to occur in the nucleus during RNA synthesis. Although 7S RNA synthesis in tomato is likely to involve DNA-dependent RNA polymerase III, by



Possible base pairing between contiguous regions of tomato 7S RNA and of PSTV as predicted by Haas *et al.*¹. Included also are possible base-paired regions in five other viroids; sequences are from refs 8, 12. Regions of three or more possible base pairs, which could theoretically form stable hybrids, are labelled A to E. There is reasonable conservation of the potential to form 7S RNA–viroid hybrids for the five boxed regions with significant variation in boxes B and C. Residue numbers given for the 5' and 3'-terminal nucleotides. G–C base pair taken as equivalent to a G–U base pair. Horizontal bars, spacings to allow maximum base pairing. CEV, citrus exocortis viroid; CSV, chrysanthemum stunt viroid; CPFV, cucumber pale fruit viroid; TASV, tomato apical stunt viroid; TPMV, tomato plant macho viroid.

produce phenotypic effects after infection, from about 2 weeks to more than 2 years. By contrast, plant viruses usually induce obvious symptoms in a few days. One of the most dramatic and damaging viroids is also the smallest: coconut cadang cadang viroid is only 246 nucleotides long, and yet it can kill coconut palms 10 to 20 metres high⁴. Expression of symptoms requires 2 years or more after infection, and is followed by certain death of palm trees within 5 years. This viroid is found only in the Philippines, where it destroys about 300,000 palms per year⁴.

Because there is no evidence that viroids can encode proteins, expression of symptoms must occur via the specific interaction of the sequence and/or tertiary structure of the viroid with one or more host components. It has been suggested by Solymosy and his colleagues⁵ that viroids exert their pathogenic effects by interfering with the normal processing of pre-ribosomal RNA in plant nucleoli. However, the new model of Haas *et al.*¹ that pathogenicity results in hybrid formation between plant 7S RNA and the viroid, offers opportunities for more specific experimental testing.

In their comparative analysis, Haas *et al.* use the sequence of potato spindle tuber viroid (PSTV), which contains 359 nucleotides, as this viroid multiplies readily in tomato plants and produces characteristic symptoms of dwarfing and epinasty. They find five contiguous regions in this viroid which could theoretically form stable hybrids with com-

analogy with its mammalian counterpart, the RNA polymerase(s) responsible for PSTV synthesis have yet to be characterized. This viroid is known to accumulate in the nucleoli of tomato cells⁶, but its site of synthesis is unknown.

Direct support for the proposed model would be provided by the isolation from PSTV-infected tomato plants of stable 7S RNA–PSTV hybrids and the characterization of the base-paired regions. This would be very difficult to achieve experimentally, but supporting indirect evidence can be obtained in other ways. Haas *et al.* find that similar hybrid complexes can be formed between the tomato 7S RNA and four other viroids which infect tomato plants⁷: citrus exocortis viroid; chrysanthemum stunt viroid; tomato apical stunt viroid; and tomato plant macho viroid. The extra comparative data for these four viroids as well as for cucumber pale fruit viroid, which also infects tomato and produces symptoms⁸, are shown in the figure.

The formation and stability of 7S–viroid hybrids can readily be tested *in vitro* by hybridization of RNA transcripts, prepared from complementary DNA clones encoding tomato 7S RNA, with purified viroids. The melting profiles of such hybrids and the determination of the exact regions of hybrid formation in each of the RNA species would provide information on the ability of such hybrids to form. There may be surprises, as two-dimensional modelling cannot take into account the stability provided to an RNA–RNA

100 years ago

THE MANATEE



THE Zoological Society have added to their living collection in the Regent's Park a young specimen of the Manatee (*Manatus americanus*), which those who wish to have an opportunity of inspecting an extremely curious form of Mammalian life should take an early opportunity of visiting. The Manatees belong to the order *Sirenia*, and are sometimes called "herbivorous Cetaceans," although it is very doubtful whether they have any near relationship to the true Whales or order Cetacea. These creatures were abundant in former geological epochs, but since the extermination of the *Rhytina*, or Steller's Sea-cow, at the latter part of the last century, have only two representatives still living, viz. the Manatee of America and Africa, and the Dugong of the Indian Ocean.

From *Nature* 39, 585; 18 April 1889.

hybrid by tertiary interactions. A good example of this is the very high stability of an *in vitro* hybrid formed between the 335-nucleotide linear satellite RNA of cucumber mosaic virus and part of the coat-protein gene encoding the viral RNA. Evidence indicated that the hybrid was formed by interaction between two short, separate blocks of nucleotides in the satellite RNA with two adjacent blocks in the coat-protein gene to produce a very stable pseudo-knot structure⁹.

Another indirect approach is to determine the sequence of 7S RNAs in other plant species where PSTV replicates and produces symptoms, and to check for the conservation of the blocks of nucleotides involved in the putative 7S RNA-PSTV hybrids. The best place to start would be with 7S RNA from two well-separated plant families, for example, another species in the family Solanaceae other than tomato, and two species from

the family Compositae which are often used for viroid replication — chrysanthemum sp. and *Gynura aurantiaca*. This approach could obviously be extended to the use of other viroids, with further fine tuning being applied through the use of different strains of the same viroid, such as PSTV or citrus exocortis viroid, where minor changes in sequence between strains can have a marked effect on the severity of symptoms in infected plants^{10,11}.

Overall, the results of such various experiments could provide support for the model of 7S RNA-viroid interaction as the primary molecular event leading to the phenotypic disease symptoms, or require its significant modification. Whatever the outcome, the new data¹ make an important contribution to the unravelling of how small viroids exert their often dramatic effects. □

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GEOCHRONOLOGY

ESR dating for the early Earth

Rainer Grun

PARAMAGNETIC centres in crystals, revealed by electron spin resonance, are used by earth scientists and archaeologists for dating samples, but because of their short mean life, the technique is restricted to the Quaternary era — the past 1.6 million years, a mere flash in geological history. Odom and Rink¹ now show that two such defects in quartz can last for at least 1.5 thousand million years. Quartz being the most abundant mineral in the Earth's crust, this study lays the foundation for an exciting new geochronometer that can be used to date numerous types of geological features over nearly the whole history of the Earth.

Electron spin resonance (ESR) spectroscopy allows the detection of paramagnetic centres in crystals, many of which are created by ionizing radiation. This effect can be used in dating studies: the mineral acts as dosimeter which records all the radioactivity of the mineral itself and its environment. The number of centres of any particular paramagnetic type is proportional to the accumulated radiation dose to which the sample has been exposed since it was last 'reset'. If one can reconstruct the dose rate the sample has received, it is possible to calculate an age.

In practice the accumulated dose is determined by the additive-dose technique: aliquots of the sample are exposed to artificial γ -radiation so that the specific ESR signal increases. Extrapolation to zero-ESR intensity yields the accumulated dose at the intersection with the dose axis. The dose rate is deduced from the chemical analysis of radioactive elements in the sample and its environment with the influence of cosmic rays allowed for.

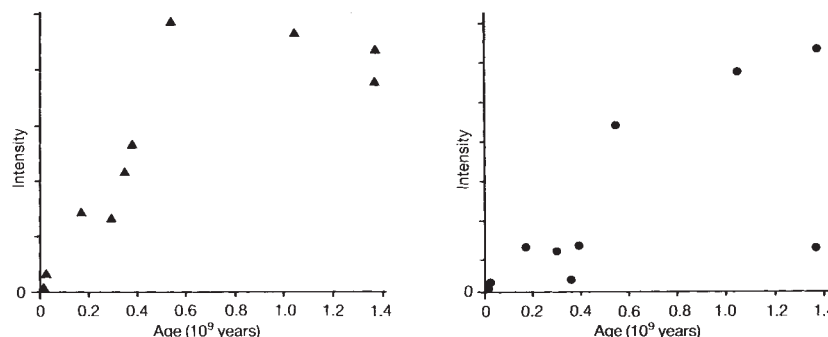
For a mineral to yield a date for an event in geological time, it must have been exposed to a process which deletes any previously stored ESR intensity. For

example, precipitation of the mineral resets the ESR clock so that speleothems, spring-deposited travertines, shells, corals or teeth can be dated. The germanium centre in quartz is bleached by sunlight, allowing the dating of dune sediments and, eventually, loess. Resetting by heating can be exploited for the dating of volcanic minerals or heated archaeological flints. And lastly, the shear stress in fault zones resets several paramagnetic centres in quartz.

As can be seen from the range of events which can be dated, ESR has a large potential for geology and archaeology. It has been applied systematically as a dating technique since 1975 when Ikeya² dated a stalactite from Akiyoshi cave in Japan. (Owing to the rapid development of the technique, the only extensive English review³ is already slightly out of date and other reviews^{4,5} have not yet been published in English.) However, when ESR is applied as a dating method as described above, the upper dating limit is set by the thermal stability of the paramagnetic centres, which persist for durations of 10^5 – 10^7 years. This has restricted ESR-dating mainly to the Quaternary period.

Odom and Rink now investigate two ESR centres which can be attributed to Schottky-Frenkel defects in the crystal lattice of quartz. These are created by elastic collisions of recoiling α -emitting nuclei with silicon and oxygen atoms. One centre is a peroxy radical, which cannot be enhanced by artificial γ -irradiation, the other is the precursor to the 'E' centre', which is a $\text{SiO}_3^{\cdot-}$ centre. The plot of the relative ESR intensity of these two centres versus the known age of the granites from which the quartz was separated (see figure) shows a clear relationship between these two parameters. This opens the possibility of dating quartz over nearly the whole history of the Earth.

Nevertheless, as the authors point out, several basic questions need to be thoroughly investigated. First, a precise measurement of the number of Schottky-Frenkel defects is still hard to obtain: the characteristic ESR lines are often very small and close to the background signal. Extreme spectrum accumulation or



Plot of relative ESR intensity of the peroxy-radical centre (left) and the E' centre (right) versus the known age of the quartz (after Odom & Rink¹).

specifically designed microwave cavities may solve this problem. Second, the precise quantities of α -particle emitting isotopes must be determined. These isotopes belong to the uranium and thorium decay chains. However, their concentration in quartz lies normally in the parts-per-billion range which is difficult to measure. The authors suggest comparing the natural ESR signal from quartz of known age with neutron-induced signals, but this approach seems unsuitable for investigation of samples of unknown age.

Third, as already pointed out by Kislyakov *et al.*⁶, the number of E'-centres per unit of radioactive elements may vary depending on the variety of the quartz under investigation. This dependency and also saturation effects would have to be studied. Lastly, the defects seem to be thermally stable for around 10^9 years. This should be confirmed, because the concentration of centres reaches a steady state in samples older than the mean life of the defects. It was previously reported⁷ that the point defects which

lead to the E' paramagnetic centre have a mean life in the range of 10^8 years (at 20 °C).

Although the investigation of these problems is not a trivial task, it seems now very likely that in the near future, ESR dating can be extended from the Quaternary to the whole geological timescale and that ESR dating will be applied to many chronological problems which could not be solved with established dating techniques. □

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CONSERVATION BIOLOGY

Nine hundred kiwis and a dog

Jared M. Diamond

INTRODUCED predators are thought to be a leading cause of the extinction of island species. Yet the evidence for this is inferential in many cases, with few or no direct observations of predation. It remains baffling how victim species can disappear so quickly, and how a predator species can eradicate a victim species completely before its own numbers collapse. Thus, the recently documented collision between one dog and a population of 900 kiwis is likely to become cited as a classic case study in conservation biology (Taborsky, M. *Notornis* **35**, 197–202; 1988).

New Zealand has lost a high proportion of its native bird species in the few centuries since Europeans arrived. Introduced weasels, cats and dogs may be responsible, but firm proof is lacking even for well-known endangered species such as the kiwi, New Zealand's national bird. The three species of kiwi are all large, flightless birds confined to New Zealand and adjacent islands, but it is not known why one (the little spotted kiwi) has vanished from the New Zealand mainland.

Until recently, the 11-square-mile Waitangi State Forest supported the largest-known and counted population of brown kiwis (*Apteryx australis*). Between June and October 1987, biologists marked some individuals of this population and followed them using radio transmitters.

On 24 August 1987, a large female kiwi was found freshly dead. Over the next 6 weeks more fresh carcasses were located, usually buried under leaves and soil. Of the 23 marked birds, 13 died during this period and were discovered through their radio transmitters. Despite the very low chances of detecting a buried kiwi carcass without the help of a transmitter, 10 carcasses of unmarked individuals were

containing kiwi feathers or possum remains. The footprints and faeces all appeared to be from the same dog. On 30 September, a German shepherd dog was found in the forest and shot. Thereafter no marked kiwis disappeared and no further dog signs were found.

How many kiwis did the dog kill? The 13 deaths among the 23 marked birds would mean 500 deaths in the whole population of 900, if marked and unmarked birds were at equal risk. In fact, the population may have suffered greater losses, because the proportion of marked birds killed was nearly twice as high outside than inside the main study of 0.5 square miles, where the biologists' presence may have deterred the dog. Five hundred killings in 6 weeks would mean about 12 kiwis killed per night. That kill rate may at first appear unbelievably large, but kiwis have a very strong distinctive smell, forage and call noisily, so are particularly vulnerable to predators.

The Waitangi incident illustrates many points relevant to the extermination of island species by introduced predators. Kiwis evolved in the absence of mammalian predators and are entirely defenceless against dogs. They breed so slowly that the Waitangi population will require an estimated 8–20 years to recover even if no such incident recurs. It took just one dog to halve the size of the largest-counted brown kiwi population in 6 weeks, yet the cause of that decline would have remained unknown but for the coincidence that some of the buried carcasses had been radio-tagged. Hence it becomes less surprising that New Zealand's abundant feral cats and weasels could have exterminated other native species without being identified as the culprits.

Bruce Coleman
The kiwi incident also illuminates the paradox of how a predator could exterminate its prey without exterminating itself first. Like most other introduced pests, dogs are 'switching predators' for which kiwis are just one of several potential prey species: this particular dog did not even eat the kiwis that it killed.

Finally, the Waitangi incident emphasizes the importance of control of introduced pests. Responsibility for the forest was recently transferred from the New Zealand Forest Service to a timber corporation, which abandoned the previous policy of keeping the forest free of dogs. As Taborsky says, "The disappearance of kiwis from other populated parts of New Zealand during the last decades underlines the general importance of the issue to the protection and management of kiwis". □

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IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

The brown kiwi is defenceless against introduced predators.

also discovered. By the end of September many fewer kiwi calls were being heard, and a dog trained to locate kiwis (but not kill them) could find none.

Tooth marks and footprints showed that all the dead kiwis had been killed by a dog, which had buried but not eaten them. All parts of Waitangi Forest that were searched yielded dead kiwis, dead possums, dog footprints, or dog faeces

New route to a sticky subject

Sharon R. Long and David W. Ehrhardt

RECOGNITION between cells or organisms is an essential component of pathogenesis, of defence and of development itself. All three processes are involved in the nitrogen-fixing symbiosis between *Rhizobium* bacteria and host plants. Specificity is one of the most prominent features of symbiotic nodule development: particular bacteria will invade and form nodules only on some host plants and not others. Because it seemed logical that surface interactions might be involved, much attention has been given to the notion that plant proteins such as lectins might specifically bind to bacteria surface determinants¹. It has been a difficult and so far elusive task to identify the bacterial surface components that might be responsible for the host-specific interaction of *Rhizobium* with plants. Now, a new study reported by Díaz *et al.* on page 579 of this issue² uses a fresh route to test specificity determinants: the genetic alteration of the composition of the root.

The authors introduce a lectin gene from pea (*Pisum sativum*) into the genome of clover (*Trifolium repens*) roots via *Agrobacterium rhizogenes* transformation. The transgenic roots still nodulate with the clover symbiont, *Rhizobium leguminosarum* biovar *trifolii*, but they display an elevated frequency of nodule formation with *R. leguminosarum* biovar *viciae*, the pea symbiont.

Host range in the *Rhizobium*-legume system exists at several levels. First, the Rhizobiaceae (*Rhizobium*, *Bradyrhizobium* and *Azorhizobium*) generally infect only plants in one plant family, the Leguminosae. Second, there are 'cross-inoculation groups' of *Rhizobium* and plants, defined by sets of bacterial strains (formerly considered species) which typically nodulate the several members of a corresponding plant set. *R. l.* biovar *viciae*, for example, nodulates peas, vetch and a few other plants, but not clover. (The study by Díaz *et al.* concerns the basis for this cross-inoculation group level of specificity.) Finally, there is a fine structure to nodulation host range, such that certain strains of a bacterial species might not nodulate all genotypes of their usual host; this behaviour is sometimes conditioned by single genes in the bacterium and the host plant.

A successful combination of host and bacteria requires compatibility at many different stages in the symbiosis, from the initial interaction to successful differentiation to nitrogen fixation³⁻⁵. The success of early *Rhizobium*-host interactions is characterized by the curly deformation of the plant's epidermal root hairs, the invasion of these root hairs by bacteria

within an infection thread (see Fig. 1) and the initiation of new cell divisions within the plant root, which eventually result in the outgrowth of a nodule. Because host specificity is often limited at these early steps, they have received most attention.

Bacteria and plants that are not in the correct cross-inoculation groups — such as *R. l. viciae* placed on clover plants — do not get as far as the infected root hair shown in Fig. 1; so there must be some barrier early in the interaction. Attachment of bacteria to the root surface might be an essential step, and it has been proposed that lectins could mediate this interaction⁶. Attachment, however, is hard to quantify, because the highly charged cell wall that surrounds all plant cells gives a substantial background to measurements of bacterial binding. Binding assays carried out using microscope observation have not been uniformly correlated with quantitative measurements using radioactively labelled cells¹. The strength of the study by Díaz *et al.*² is that such a problematic assay is not involved; a discrete change was made in host cell determinants using the plant's own cellular machinery, and this change



FIG. 1. Successful infection of *Rhizobium* into many legumes is characterized by formation of an infection thread in an epidermal cell. Normally, straight root hairs grow into a curled form and are invaded by *Rhizobium* of the correct cross-inoculation group. No defence reaction occurs in the root-hair cell; instead, the bacteria penetrate to the main body of the root within the actively elongating, branching infection thread whose wall is synthesized by the plant cell. The dynamic process must involve highly localized and directed bacterial functions, and a complex cell-wall growth response in the plant. Genetic studies on *Rhizobium* indicate that specificity determinants are an intrinsic part of the mechanisms directing these active plant responses.

leads to a marked alteration in nodulation phenotype. It is noteworthy, however, that although the transgenic clover roots studied here gain significant nodulation functions, the gain of function is not complete. Nodules form infrequently, and are often abnormal in morphology, suggesting that although the pea lectin represents one primary limitation or screen for symbiotic partners, there are others.

That the determination of host range involves multiple components and steps is also now indicated by studies of bacterial symbiotic genes. Numerous bacterial genes have been identified as affecting nodule formation, and these fall into several categories³⁻⁵. Some, for example *nodABC*, are highly conserved, both in DNA sequence and in function, and seem to be central to the stimulation of nodule formation on all plants. Another type, such as *nodH* in the alfalfa symbiont *R. meliloti*⁷, are unique to bacteria of a particular nodulation specificity. Other genes, including *nodFE*, are conserved among different *Rhizobium* strains in terms of their nucleotide sequences, but they nevertheless seem to affect host range. The *nodD* regulatory genes even seem to combine universality and host selectivity: although the ultimate regulatory effect of *nodD* — the activation of transcription of nodulation genes — is parallel in various *Rhizobium* strains, the spectrum of plant-derived inducer molecules which stimulate this effect varies for *nodD* genes derived from different *Rhizobium* species⁸.

The genes known to be present in *R. l. trifolii* and *R. l. viciae* are remarkably similar (see Fig. 2), yet the bacteria nodulate different hosts⁹. Experiments to analyse this have revealed unexpected properties of some host-range genes. *R. l. trifolii* containing a mutated *nodF* or *nodE* gene is converted to behave more like *R. l. viciae*, for example — it broadens its host range to interact with pea, and becomes less efficient on its original host, clover⁴. In general, individual host-specific nodulation genes seem to affect efficiency or frequency, but do not seem to be absolutely required. Thus, whereas host range itself is a strict phenotype, mutation of individual bacterial genes controlling host range gives leaky phenotypes. Such observations, together with studies in several laboratories of double mutants, have led to the widely discussed proposal that the host-range phenotype results from the additive effects of multiple mechanisms, which may exist in differing combinations among various host-microbe pairs, not in mutually exclusive sets. The picture that has emerged from genetic studies, then, is that host range is not controlled by a single locus, in which one key is matched to a lock, but is more likely to be combinatorial.

Díaz *et al.*² demonstrate that a lectin can

FIG. 2 Map of nodulation genes in two biovars of *Rhizobium leguminosarum*, *a*, by *viciae*, which infects pea, vetch, lentil and related plants; and *b*, by *trifolii*, which infects clovers. The genes have been established by transposon mutagenesis, sequencing and some protein product analysis^{4,5}. Asterisks indicate positions in which various researchers believe further genes will soon be defined. Those genes designated 'common' are functionally interchangeable without an effect on host range; in particular, *nodABC* seem to stimulate host cell divisions and nodule formation, and are also required for root-hair deformation³. These common genes do not function well without accompanying host-specific nodulation genes, such as *nodFE*, which appear critical for proper root-hair curling and infection^{4,5}. An example of a fine-structure host range gene is *nodX*, found in some but not all *R. l. viciae*; without *nodX*, *R. l. viciae* strains are restricted to nodulating only certain genotypes of pea⁵.

be important in this process. Whether changing the lectins expressed in other host plants can similarly affect infection by *Rhizobium* remains to be seen. The two bacteria which nodulate pea versus clover are more closely related than most pairs of *Rhizobium*. Those which are less closely related may have more dramatic limitations to nodulation on their heterologous hosts. It would not be surprising if different host/symbiont combinations in varying isolation evolved exclusivity and compatibility to different degrees and along slightly different avenues.

The molecular role of any receptor, of course, remains difficult to study until it is known to what it binds. This remains a sticky point in research on *Rhizobium*-legume systems. If plant lectins are receptors for bacterial carbohydrates, it may be that the relevant sugars are sweetening up a surface component not identified to date. For example, researchers working on the genes which control several types of extracellular polysaccharides in *Rhizobium* find that eliminating or altering surface components such as the acidic exopolysaccharide⁹ or the lipopolysaccharide¹⁰ prevents successful invasion, but allows formation of uninfected nodules in a correct host-specific manner. Therefore, these carbohydrates do not seem to correlate with host range, although it remains possible that there is redundant information in several surface determinants. Identification of specific bacterial molecules interacting with host components will be essential before the issue of how lectins affect the symbiosis is resolved.

The availability of transgenic roots with altered nodulation properties will allow genetically tailored microbes and plants to be paired with each other for specific mechanistic tests. It is of interest, for example, to test bacteria altered in known nodulation genes on transgenic roots with host-range changes that are complementary. Expression of lectin genes in heterologous backgrounds may help to determine exactly where the lectin is expressed and localized in transgenic plants, for example, and under what circumstances its functional properties are preserved. Previous studies on pea have shown that lectin binding of *R. l. viciae* can be assayed only under manganese limitation of the

Host plant specific	"Common"	Host genotype specific
* NM L EF D ABCIJ	X	<i>R. l. viciae</i>
* NM L* EF D ABCIJ	*	<i>R. l. trifolii</i>

bacteria¹¹: do the transgenic clover/pea lectin roots bind their new *R. l. viciae* symbionts in this manner?

Finally, how are host-range functions coupled with the action of the common nodulation genes? Genes such as *nodABC* drive the plant to begin cell divisions, and are also required for root hair curling and invasion, but they do not have a productive effect on a host plant unless they are accompanied by the proper host range genes. Bacteria with normal *nodABC* genes, but lacking a host-range gene, for example, often cause the plant to exhibit incomplete or atypical root-hair deformations. If host-range genes are additive in effect, then perhaps they act by modifying a central function or functions provided by the common *nod* genes, increasing their efficiency. There is new experimental support for this model¹². In addition, host-range genes might also act by directing the location and timing of the action of *Rhizobium* on its target; by providing access for a molecular signal or a cell to a site of action; by actively modifying the bacteria's immediate molecular environment within the plant cell wall or membrane; by preventing a host defensive reaction; or by some combination of these. Given that the transfer of the pea lectin can change the responsiveness of clover roots to *Rhizobium*, it may be possible to test whether the function of this lectin is simply in attachment, or whether it acts through more complicated, possibly more active, mechanisms. □

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Long sight

LAST week Daedalus devised his 'Space Spider'. This ingenious spacecraft propels itself by melt-spinning and ejecting a fine optic fibre. Cunningly, the fibre is made of a lasing glass, excited by the sunlight of space. It amplifies light pulses as they traverse it, and forms an active optical communication link back to Earth.

Daedalus now reckons that his space-borne light guide should be a powerful research tool in its own right. Thus it could form an astronomical interferometer of unprecedented resolution. The light from a stellar source spreads out in a wide wavefront. When two separate sections of the wavefront are recombined, traditionally by means of a pair of mirrors, the resulting interference pattern can be decoded into an image of the source. It is hard to give such an interferometer a mirror separation greater than about ten metres, so it can only just resolve the diameters of the nearer, bigger stars. But an active space-borne optic fibre could recombine sections of wavefront millions of kilometres apart.

Daedalus envisages a command spacecraft with two fibres extending out from it, their far ends anchored to two observation platforms. Each platform would use a stabilized telescope to image light from the chosen astronomical object onto the end of its fibre. The light from the two telescopes would flow down the fibres and interfere at the central command craft. If the orbits of the telescopes were chosen to vary the distance and angle between them, the changing interferogram would slowly accumulate an aperture-synthesized image of the object. A source a thousand light years away could be resolved to a few kilometres!

More boldly still, Daedalus wants to use his space-borne fibre to detect gravity waves. These energetic but elusive phenomena are emitted by massive moving objects like big, short-period binaries, collapsing neutron stars, supernovae, and black holes in the act of swallowing a star. Even gravity waves from the early days of the Universe may still be echoing around. They should shake an optical interferometer as they pass through it. Terrestrial interferometers have so far failed to detect them: but Daedalus's space-borne fibre interferometer should be mightily more sensitive. Its optical length would only change in second order, but the shift of the interference pattern should still be very clear. Gravity waves would also deform the fibre slightly as they passed through it. The birefringence thus induced would be tiny indeed — but a light pulse traversing a million kilometres of such a fibre should suffer detectable changes of polarization. Much exciting astrophysical mayhem, undetectable by optical or radio emission, should thus be revealed in all its gravity. David Jones

Leucine zipper motif extends

SIR—Landschulz *et al.*¹ have recently proposed that the 'leucine zipper' is characteristic of a new category of DNA-binding proteins. In this hypothetical structure the side-chains extending from an heptad repeat of leucine residues over a distance covering (at least) eight turns of an α -helix, interdigitate with those on a similar helix of a second polypeptide, thus facilitating dimerization. The Fos and Jun transforming proteins both contain such a motif and Landschulz *et al.* speculate that it plays a role in the heterotypic complex these proteins can form¹.

We report the presence of the same motif in proteins that are not DNA-binding. As shown in the figure, the fusion (F) glycoproteins of paramyxoviruses each have a leucine-zipper motif, which is in an α -helical structure situated 4–11

Virus		Ref.
MPV	N L G N A I A K L E D A K E L L E S S D Q I L R	7
RV	N L W N A V T K L E K A K D L L D S S D L I L R	8
CDV	N L G N A L K K L D D A K V L I D S S N Q I L E	9
HPV 1	N L A S A T N F L Q Q S K I Q L M K A K A I I S	10
HPV 3	E L N K A K S D L E E S K E W I R S N Q K I D	11
Sendai	N L A D A T N F L Q D S K A E L E K A K K I L S	12
Mumps	E L S K V N A S L Q N A V K Y I K E S N H Q L Q	13
SV 5	N L A A V N K S L S D A L Q H L A Q S D T Y L S	14
NDV	E L G N V N N S I S N A L D K L E E S S K L D	15

Comparison of leucine-zipper motif in F proteins of different paramyxoviruses. Leucine heptad repeats are marked by asterisks. Viruses: MPV, measles; RV, rinderpest; CDV, canine distemper; HPV 1, human parainfluenza 1; HPV 3, human parainfluenza 3; SV 5, simian 5; NDV, Newcastle disease.

amino acids from the transmembrane area. In some cases, isoleucine is substituted for one of the leucines, but Kouzarides and Ziff³ have shown that this does not affect the interaction of Fos and Jun. Further analysis of the proposed structure reveals that at position 4 alongside the leucine, there is always a small uncharged amino acid, whereas charged amino acids are distributed in the other five positions.

Studies with influenza haemagglutinin⁴ and the glycoprotein of vesicular stomatitis virus⁵ have shown that after their synthesis in the endoplasmic reticulum, these glycoproteins must oligomerize in order to be transported to the cell surface via the Golgi complex. Conceivably, the same is

true for the F glycoproteins of paramyxoviruses, in which case dimer and tetramer formation may be mediated by the leucine zippers. The F glycoprotein of Sendai virus is a tetramer when isolated under non-denaturing conditions⁶.

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Stereo problem

SIR—I have frequently suffered frustration because of the reversed depth and handedness of stereo-images when viewed directly from the printed page, especially when other clues such as the size of components or their shading are adjusted to give parallel depth information. Like most people, I view such images with crossed visual axes. The diagram in Vance Tucker's letter (*Nature* 337, 605; 1989) suggests a simple solution.

If one member of a stereo-pair were to be flanked on either side by identical copies of the other stereo-pair, as in Tucker's diagram, then a reader could see the correct depth and handedness by looking at, say, the left pair with uncrossed visual axes and at the right pair with crossed axes. The same procedure could also be used with projected images from slides, films or overhead projectors, although given that many individuals who view close stereo-pairs with uncrossed axes view distant pairs by the crossed method, it would probably suffice to project only the 'crossed' arrangement. Unfortunately, in my experience it is more usual for the uncrossed images to be projected or printed (for example, the stereo-pairs on pages 618 and 619 of the same issue of *Nature*), causing confusion to most direct viewers.

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Stop taxonomists

SIR—Replying to complaints¹ about the number of changes in biological nomenclature, Hawksworth suggests² that "international peer reactions are the most likely prospect for restricting unwelcome changes...". Unfortunately, this restriction seems unlikely to happen if the peers are fellow taxonomists.

One difficulty stems from some of the general assumptions of taxonomists: even Hawksworth's distinguished predecessor, G.C. Ainsworth, held that more is better.

He wrote³: "the very welcome upward trend in the proportion of new combinations indicates that increasing attention is being paid to revision".

Biologists need good taxonomy, with each name unambiguous and reasonably constant. However, in their enthusiasm, taxonomists make frequent changes and revisions of these changes that cause confusion to other biologists who use the names. This is nothing new; it has been going on for years and it is time it stopped.

This conflict of interest between taxonomists, who want to change the names of organisms, and other biologists, who do not, could be resolved. Taxonomists could continue publishing their findings, as now, but international standing commissions could review these publications every few years and issue lists of official names. These names would hold until the next list was issued, so that names would be standardized over periods of several years.

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Carbonatite origin and diversity

SIR—A recent paper¹ and an accompanying News and Views article² explained the origin and diversity of carbonatites by production of primary carbonatite magma in the mantle, followed by fractional crystallization. Gittins² considers the experimentally produced carbonatite melt of ref. 1 to be particularly relevant to the problems of carbonatite magma genesis because it contains the oxides required to crystallize the silicate, phosphate and oxide minerals that are commonly associated with the carbonate minerals that form the majority³ of carbonatite rocks.

In contrast, Gittins² asserts that the carbonate liquids produced by immiscibility in our experimental studies^{4,5} are too poor in non-carbonate components to be considered relevant to natural systems. Twyman and Gittins⁶ previously postulated a mildly alkaline olivine siltite as a suitable parent magma; the proposed composition of this magma is listed in the table, together with the carbonatite melt composition of ref. 1 and immiscible silicate/carbonate liquid pairs from refs 4 and 5 and our new work (in preparation).

Contrary to Gittins' statement, some of the carbonate liquids produced experimentally in ref. 4 contained significant amounts of non-carbonate components. Although the FeO and MgO contents of these carbonate liquids are quite low, this simply reflects the fact that the starting bulk composition was very poor in these

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Composition of carbonatite melts and conjugate silicate liquids

Oxide	1	2	3	4	5	6	7	8	9	10
SiO ₂	2.94	6.56	4.50	1.60	6.43	9.33	44.46	32.38	42.12	32.51
TiO ₂	0.45	1.66			0.94	0.81	0.73		2.28	1.70
Al ₂ O ₃	1.95	1.09	0.40	0.36	0.80	0.91	14.68	8.62	14.79	7.76
FeO _{tot}	4.61	10.66	2.30		4.18	4.12	5.41		7.92	6.65
MgO	14.19	3.68	0.92		1.00	10.93	0.65		0.91	10.49
MnO		0.25			0.23				0.18	
CaO	21.29	34.50	31.59	55.46	37.16	29.19	11.13	42.92	12.77	16.14
Na ₂ O	4.99	5.74	16.35	0.28	7.27	3.56	11.07	6.22	8.91	6.60
K ₂ O	0.35	2.41	5.17		1.73	2.34	5.73		3.53	3.34
P ₂ O ₅	0.48	5.92	1.21		0.56	2.19	0.23		0.12	1.41
CO ₂		28.17								
Total	51.49	100.00	62.44	57.70	60.30	63.37	94.09	90.14	93.53	86.60

- (1) Carbonatite melt produced in equilibrium with a peridotite source (column 3 of Table 1 in ref. 1). (Total includes Cr₂O₃ = 0.22.)
- (2) Carbonatite parent magma of Twyman and Gittins⁶.
- (3) Immiscible carbonatite melt FH24 of Freestone and Hamilton⁴, $P = 7.6$ kbar, $T = 1,100$ °C.
- (4) Immiscible carbonatite melt KH11 of Kjarsgaard and Hamilton⁵, $P = 5.0$ kbar, $T = 1,250$ °C.
- (5) Immiscible carbonatite melt BK208 of Kjarsgaard and Hamilton (unpublished), $P = 5.0$ kbar, $T = 1,000$ °C.
- (6) Immiscible carbonatite melt BK193 of Kjarsgaard and Hamilton (unpublished), $P = 6.0$ kbar, $T = 1,200$ °C.
- (7) Conjugate silicate liquid to (3).
- (8) Conjugate silicate liquid to (4).
- (9) Conjugate silicate liquid to (5).
- (10) Conjugate silicate liquid to (6).

components. The distribution of elements between immiscible liquids is strongly affected by pressure, temperature and the bulk composition of the system¹, with compositional effects being particularly important (see Fig. 6 of ref. 4). Kjarsgaard and Hamilton⁵ used compositions containing only five components (SiO₂–Al₂O₃–CaO–Na₂O–CO₂), so the immiscible carbonate liquids produced could not contain FeO, MgO, P₂O₅ and the like. Gittins was correct to point out that these melts were poor in SiO₂ and Al₂O₃ (see column 4 of the table), but again this could be the result of pressure, temperature and composition. Our more recent (unpublished) results show that the addition of TiO₂, MgO, FeO, F and P₂O₅ ensures that these components occur in the immiscible carbonate melt, and also that the addition of these components affects the partitioning of Al₂O₃ and SiO₂ between immiscible melts such that the carbonate liquids contain noticeably higher contents of these two elements.

The table shows that we have produced, by immiscibility, carbonate liquids whose compositions compare favourably with those considered to be "realistic carbonatite parent magmas" by Gittins (compare columns 5 and 6 with columns 1 and 2). Furthermore, the conjugate alkali silicate liquids produced in these experiments (compare columns 9 and 10) have compositions that are suitable as parent magmas for the attendant alkali silicate rocks found in carbonatite complexes.

Gittins² suggests that the next step in testing the primary-melt model of ref. 1 is to examine rare-earth element concentrations. Hamilton *et al.*⁷ have made this test for an immiscible silicate/carbonate system, using lavas from Oldoinyo Lengai as

starting materials. Rare-earth element concentrations for the Oldoinyo Lengai phonolite and natrocarbonatite⁸ are used in ref. 7 to calculate a set of natural rock partition coefficients, and these compare very well with those derived from the experiments at 3 kbar and 1,050 °C.

Any debate on carbonatite magma genesis should include proper consideration of the viability of the immiscibility hypothesis. This process, in conjunction with fractionation, can convincingly account for both the major- and trace-element contents of carbonatites as well as the associated alkali silicate rocks.

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GITTINS REPLIES—The proponents of liquid immiscibility as the explanation for carbonatite magmas consider the experimental demonstration that various carbonate and silicate liquids are immiscible up to at least 8 kbar pressure to be proof that the carbonate and silicate liquids separated from a common parent magma, whereas I maintain that one must demonstrate not only the existence of liquid immiscibility but also a derivative relationship. In my view this requires the formation of a single quenchable liquid from the two immiscible liquids. Such a liquid might be the hypothetical CO₂-rich nephelinite (or phonolite) that is invoked by Le Bas⁹. If it exists in nature it should be possible to make it in the laboratory.

Until this is done we can only examine the natural rocks for evidence of a CO₂-rich nephelinite that has fractionated to a stage just short of single-liquid instability,

and ascertain what sort of rock it is. We find no such rock — only simple olivine nephelinites and nephelinites.

The postulated parental magma of ref. 6 is not completely correct (for example, the P₂O₅ content is too high) but it is a useful beginning. Some of Kjarsgaard and Hamilton's immiscible carbonate liquids are similar but they and the K_D values of the table are relevant to the problem only if carbonatite magma separates immiscibly from a silicate parent (which is not yet proved) and if the contents of rare earths and other elements such as Nb, P, Sr, Ba, F and Cl are sufficiently high in the parent silicate magma for partitioning to produce the characteristic carbonatite element concentrations. In saying that the next step in testing the primary melt model of ref. 1 would be to examine the rare-earth element concentrations, I was simply making the point in my News and Views article³ that unless the liquid of ref. 1 contains the necessary major- and trace-element concentrations it is not a viable parental carbonatite magma. The fact that Hamilton *et al.* have successfully partitioned rare earths into a carbonate liquid at the expense of an immiscible silicate liquid is interesting, but irrelevant to evaluating ref. 1.

I might also point out that if the experiments in ref. 7 used only CO₂ as a transporting fluid they are of limited value because the mobility of CO₂ is restricted to a depth corresponding to 27 kbar, the pressure at which silicate carbonation reactions fix CO₂. Something capable of transporting elements at greater depths is required, and our (unpublished) work suggests that fluorine plays a major part in this and in just about every other aspect of carbonate liquid systems.

Carbonate/silicate liquid immiscibility exists. But is it merely a physical property of silicate and carbonate magmas or does it control their genesis? Do these magmas separate as conjugate liquids from a single mantle-derived parent, or are they each the result of separate mantle-melting events? Is immiscibility a relatively late-stage, upper-crustal process of minor petrological significance, or the principal process by which carbonatite magma is generated?

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In pursuit of the peculiar

P.V.E. McClintock

Methodological Aspects of the Development of Low Temperature Physics 1881–1956: Concepts out of Context(s). By Kostas Gavroglu and Yorgos Goudaroulis. *Kluwer Academic*: 1989. Pp. 178. Dfl.125, \$67, £36.

PROGRESS in science often arises through a fruitful interplay between experiment and theory. New experimental observations — made possible, perhaps, by some advance in instrumentation — may fail to agree in all respects with theories constructed to account for related phenomena; those theories then have to be modified, leading to fresh insights and, perhaps, to new predictions which can then in turn be tested. But what happens when the discrepancy between observation and expectation is so gross that the existing theoretical framework simply cannot, by any stretch of the imagination, be modified or extended to accommodate the new information? In their book, Gavroglu and Goudaroulis attempt to answer the question through an historical study of low-temperature physics.

Both of the authors are active in theoretical atomic and elementary particle physics. Given this background, it is interesting to note that, in seeking the “most peculiar” phenomena on which to base their study — admittedly a subjective judgement — they eventually “were amazed to realize that neither the phenomena of the very small nor those of the very large could compete with the phenomena of the very cold”.

The phenomena in question are superfluidity and superconductivity, and they certainly are peculiar. Each of them involves the onset of a form of frictionless flow below a critical temperature. In the case of helium, the liquid acquires the ability to flow easily through tiny orifices of vanishingly small dimensions or, even more astonishingly, to climb out of any open-topped vessel in which it is placed: in fact, it behaves as though its viscosity was zero, whence the appellation superfluidity. The frictionless flow of the electron gas in a superconducting metal, in many ways a similar phenomenon and equally unexpected at the time of its discovery, means that an electron current can flow forever around a closed loop of conductor without needing a driving voltage to maintain it; the technological implications of superconductivity, particularly if large current densities can be achieved at relatively high temperatures, have been well publicized in recent months.

In outline, the story of the discovery of superfluidity and superconductivity is a familiar one. It starts, in effect, with the liquefaction of helium — the last of the so-called permanent gases to succumb — by

Kamerlingh Onnes at Leiden in 1908. Once liquid helium was available for use as a cryogenic fluid, experiments went ahead on a wide range of materials at very low temperatures. In 1911, Onnes reported an exceptionally odd and, as it turned out, historic observation that the electrical resistance of mercury apparently fell abruptly to zero (that is, the

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Kamerlingh Onnes — historic observation. onset of superconductivity) as it was cooled through a transition temperature of about 4.2 K.

Although liquid helium continued to be used as a cryogen to cool a variety of experiments at Leiden and later at several other laboratories, and although many researchers were aware that it was an unusual liquid, its really remarkable property (of superfluidity) was not appreciated until surprisingly late, in 1938, with the famous Volume 141 of *Nature* in which workers in Cambridge, Moscow, Oxford and Kharkov reported almost simultaneously a whole range of phenomena leading in different ways to the same conclusion: that liquid helium below 2.17 K is a superfluid. The authors describe the history of these events in considerable detail,

mainly through reference to the literature, but also on the basis of their visits to Leiden and other centres of low-temperature research, and in the light of their discussions and correspondence with survivors from the latter part of the era in question, and with their collaborators and successors. They concentrate mainly on the period 1908–1956, but also provide valuable background on earlier years and an outline of developments after 1956.

The discovery of superconductivity certainly occurred “out of context”. It came while the quantum theory was in its infancy, before the development of quantum statistical mechanics, and it far preceded the advent of those theories of the solid state which were to be a prerequisite for its proper understanding. Superfluidity also arrived “out of context” but, coming more than a quarter of a century later, somewhat less so. The authors discuss in fascinating detail the twists and turns of the argument and the interplay between experiment and theory which led, in the end, to reasonably satisfactory understanding of the two phenomena by the mid-1950s. They could also have pointed out, however, that in the case of superconductivity, the resultant theory — the Bardeen–Cooper–Schrieffer (BCS) theory — was a good deal fuller, more fundamental and generally more satisfactory than that for helium (an uneasy co-existence of the early ideas of London, Tisza and Landau, together with the later unifying contributions of Feynman and others).

The approach taken by Gavroglu and Goudaroulis in analysing these events relies on their idea of “concepts out of context(s)” wherein a paradoxical situation is created by the initial attempts to describe a new phenomenon, precisely because it is incompatible with prevailing theory. The resolution of the paradox involves the development of a new theoretical framework, providing a context within which the phenomenon in question will be expected, rather than unexpected. Few scientists are likely to disagree with such an interpretation, which, stripped of the authors’ jargon, is a seemingly obvious one.

Gavroglu and Goudaroulis tell us a tale of considerable intrinsic interest involving technical ingenuity, astonishing discoveries, imagination, insight, bitter arguments between proponents of conflicting theories and, ultimately, triumph in terms of scientific understanding. The story is carefully told, with copious annotations and references to the original literature. There are some obvious lacunae, however, particularly in relation to historical perspectives on superfluid helium. For example, in the course of their description of the early controversy surrounding London’s seemingly outrageous suggestion that Bose–Einstein condensation (where, in

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an ideal gas at a low enough temperature, particles 'condense' into the zero momentum ground state) might also occur in liquid helium, the authors do not mention that London was eventually proved correct by the neutron-scattering experiments of Eric Svensson and co-workers at Chalk River. Similarly, they provide an account of the Landau critical velocity paradox — Landau having predicted a critical velocity for the onset of frictional effects in superfluid helium that was several hundred times larger than the critical velocity actually observed in the early experiments — and of Feynman's resolution of the paradox in terms of dissipation through vortex production at lower velocities. But they forbear to mention that Landau was actually correct all along; the critical velocity for the onset of non-vortex dissipation, subsequently measured at Lancaster, was in excellent quantitative agreement with the original Landau prediction.

The saga is likely to be of interest to historians and philosophers of science. It will certainly provide fascinating reading for physicists, and in particular for all those involved in research at low temperatures. □

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Greater clarity on crystals

Alan L. Mackay

Introduction to Quasicrystals. Edited by Marko V. Jarić. *Academic:1988. Pp.285. £31.50, \$50.*

MOST textbooks on crystallography and solid-state physics begin with the (still undoubted) proposition that, in an infinite crystallographic lattice, lattice points, all of which have identical surroundings, cannot have fivefold symmetry. Their authors also assume, and would like to prove, that the most stable state of an assembly of identical units is a crystal. They fail to prove this proposition rigorously, perhaps because it may not be true, and then continue with the physics of crystals.

Crystallographers have long been aware of the existence in crystals of molecules and clusters of icosahedral symmetry, and of the frequent incompatibility of the local symmetry of such groups with the limited point-site symmetries available in the 230 crystallographic space groups. Fivefold or icosahedral symmetry in extended crystals was universally assumed to be impossible. It was, therefore, a tremendous surprise when, in 1984, Dan Shechtman and his colleagues at the National Bureau of Standards in the United States reported that extended regions of a rapidly quenched alloy, of composition about $Al_{63}Mn_{37}$, showed electron-diffraction patterns with sharp spots and with full icosahedral symmetry. Since the appearance of their paper, some 600 further papers on the new structures, now called 'quasi-crystals', have been published. With the present volume we now have the first book on the topic, presenting the material in more digestible form (a convenient basic compendium of papers, with commentary, had earlier been produced by Steinhardt and Ostlund).

There is nothing actually wrong with the crystallographic rules which we knew before 1984, but we have had to look much more closely at the gaps between them. Curiously, the phenomenon turns on unobserved phase angles. In X-ray diffraction from crystals, the Braggs' insight was to see that each spot in the diffraction pattern corresponded to a sinusoidal density wave of scattering matter and that the crystal could be considered as the linear superposition of these. The amplitudes of the waves are easily recorded but their phases are not. It is the art of X-ray crystal structure analysis to recover them.

The 230 space groups prescribe the symmetry relationships between the phases from the assumption of crystallinity. We are still free to make structures

with some particular symmetry for the amplitudes but no necessary symmetry for their phases, which may also vary from place to place in the material. People had assumed that any structure that gave diffraction patterns with sharp spots must be periodic. It has now been shown that materials with two incommensurate periodicities combined in the right way (by being a section through a higher-dimensional lattice), or being quasi-periodic in the strictly mathematical sense of the term, also show sharp spots.

It turns out that quasi-crystals exploit this unobservability of the phases. The diffraction effects may have icosahedral symmetry but that does not mean that there are fivefold axes in the structure. Since about 1974, the Penrose tiling has been known as a mathematical game and it was immediately seized upon as a patterning system which would produce diffraction phenomena like those observed. The Penrose tiling exemplified the extremely ingenious compromise found by nature between the demands of local icosahedral order and of long-range order.

Since 1984 the matter has become immensely more complicated. In the 1930s crystallographers discussed all kinds of structures between perfect crystals and glasses, and indeed the possibility of icosahedral textures was explicitly mentioned. But recently the initiative has passed to the solid-state physicists, who have attacked the area in massive strength.

In this volume, the first of a series entitled "Aperiodicity and Order", Marko Jarić has collected together six essays which lay out the new subject coherently. To start with, David and Clara Shoemaker describe the local icosahedral coordination often observed in metallic crystals, some of great complexity, which lays the foundation of local order — one of the consolations of ageing is that one can enjoy seeing their stereo-pairs without a stereoscope. Michael Widom looks at short- and long-range order more generally, introducing the intriguing relevance of four-dimensional polytopes as poly-tetrahedral structures with local icosahedral coordination, and discussing how they may be decurved back into the normal three-dimensional world in which the quasi-crystals must exist. Robert Schaefer and Leo Bendersky then provide a metallurgical setting for the occurrence of quasi-crystalline phases.

Per Bak and Alan Goldman begin on the new quasicrystallography, showing how structures with the necessary diffraction properties can be derived as projections of lattice structures in higher dimensional space. The phases with icosahedral diffraction symmetry can be derived as three-dimensional sections through a six-dimensional hypercubic lattice. Accordingly, diffraction spots can be assigned six-

integer indices with respect to this lattice; there is an analogy with the way in which ordinary hexagonal crystals are given four indices with respect to a four-dimensional lattice.

The remaining two chapters — "Stability and Deformation" by O. Biham, D. Mukamel and S. Shtrikman, and "Symmetry, Elasticity and Hydrodynamics in Quasiperiodic Structures" by T.C. Lubensky — take the reader into the field of solid-state physics rather than conventional crystallography. It is clear that crystallographers will have to absorb this new material. It might even be said that while they have been away capturing large areas of biology, the physicists have moved in and occupied the central camp of crystallographic theory. Crystallographers have become the victims of their own success (based on crystal structure analysis); phases, such as liquid crystals, which were somewhat disdained as being

inferior, have been taken over by solid-state physicists who have applied sophisticated theoretical analyses which crystallographers can hardly match.

Altogether, the quasicrystal episode has been of the greatest interest. The whole area of crystal symmetry — which is often quoted as being one of the most tightly sewn up with a beautiful and complete theory — has been broken open by an experimental observation, resulting in a mass of exciting new theories, many of which can be connected with actual materials. We can look forward to further books on the subject, where the new ideas will be laid out with increasing clarity and simplicity. The opening chapters of all volumes on crystallography and solid-state physics must henceforth begin differently. □

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Electronified

D. G. Pettifor

Introduction to the Modern Theory of Metals. By Alan Cottrell. *Institute of Metals, 1 Carlton House Terrace, London SW1Y 5DB/Old Post Road, Brookfield, Vermont 05036:1989. Pp.260. £35, \$73.50.**

THE classic textbooks by Hume-Rothery and Raynor on the electron theory of metals were published soon after the end of the Second World War. They were imbued with the optimism, prevalent at that time amongst scientists, that science could win the peace just as it had helped win the war. In response to the query why metallurgists should know about quantum theory, Raynor writes in his 1947 preface: "If the metallurgists of [Britain] do not learn about electron theories, they may discover, too late, that industry in other countries has benefited from knowledge of *modern theories* and is developing new alloys".

This belief in the power of electron theory to place the metallurgical industry on sound scientific foundations did not seem misplaced. Hume-Rothery's pioneering work on the solubility limits and crystal structure of copper, silver and gold alloys had been given a simple theoretical

explanation by Jones in 1934. Experimentally, the maximum solubility of polyvalent elements such as zinc, aluminium or tin in the monovalent noble metals was found to occur at an average number of valence electrons per atom of approximately 1.4 (assuming a valence of 1, 2, 3 and 4 for copper, zinc, aluminium and tin respectively). Jones pointed out that this was very close to the number of electrons per atom which is required for the spherical free-electron Fermi surface to make contact with the Brillouin zone boundary corresponding to the face-centred cubic lattice of the noble metals. Further solute atoms would push electrons into the high-energy states across the gap at the zone boundary. This is energetically unfavourable and Jones predicted the face-centred cubic lattice would undergo a transformation to the body-centred cubic lattice, as is observed experimentally.

Alas, this optimism in the usefulness of modern theory for alloy development was dashed ten years later when, in 1957, Pippard discovered that the Fermi surface had *already* made contact with the Brillouin zone boundary in elemental copper, even though the electron per atom ratio was only one. The approximation of treating copper's valence electrons as a gas of free electrons which are only weakly perturbed by the ionic lattice — a problem which theorists could solve — was simply too crude. In practice, the full 3d electronic shell in copper is not inert but hybridizes strongly with the valence sp band, thereby giving copper its characteristic colour and high cohesive energy. At the time, however, controversy surrounded how best to treat the valence d electrons in transition and noble metals — whether to regard them as localized on their parent atoms or itinerant. As Hume-Rothery wrote in the preface to the 1962 fourth (revised) re-

print: "The work of the last ten years has emphasized the extreme difficulty of producing any really quantitative theory".

The appearance of Alan Cottrell's new book *Introduction to the Modern Theory of Metals* is indicative that the theoretical physicists have now put their house in order and can offer the metallurgist reliable quantitative insights into the world of metals at the atomic level. The magic touch, which he displayed in his earlier textbooks on metallurgy, is still very much in evidence as he describes the developments of the past 30 years that have placed the modern theory of metals on such a firm foundation. Coming from outside the field of electron theory himself, he offers a broad vision and a clarity of purpose.

The book begins with a discussion of the fundamental question 'What is a metal?'. The recent discovery of high-temperature superconductivity in ceramic oxides has driven home the fact to a wide audience that Wilson's original distinction between metals and insulators in terms of energy bands and Brillouin zones is fatally flawed. Cottrell stresses that physicists and chemists are now converging in their views on highly correlated systems — Mott's famous criterion for the metal-insulator transition being similar to a pre-quantum treatment of the problem as a polarization catastrophe by Herzfeld and Goldhammer, and Anderson's model of high-temperature superconductivity building on Pauling's ideas of the resonating valence bond. One-electron energy-band theory is, however, shown to be well justified for normal metals in which the electrons are less highly correlated. Excellent chapters introduce the ideas of quasiparticles, pseudopotentials and density functional theory which are at the heart of modern quantitative electron theory. Others discuss the cohesion and structure of simple metals, transition metals and their surfaces, and recent theories of alloy formation.

Introduction to the Modern Theory of Metals will be essential reading for students of metallurgy and solid-state physics. The mathematics is kept to a minimum within the main text, the more detailed working and theory being relegated to appendices, so that in Cottrell's hands even the mathematically weakest student should be able to follow the physical arguments. Research workers wishing to understand recent advances in the electron theory of metals will also find the book invaluable. With industrial laboratories the world over engaging electron theorists to help them in the search for new and better alloys, Raynor's optimism no longer appears to be so misplaced. The electron theory of metals has come of age. □

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* To coincide with the publication of Cottrell's book, The Institute of Metals has reprinted three titles on the same or related topics — *Atomic Theory for Students of Metallurgy*. By W. Hume-Rothery and B. R. Coles. Pp.428. Pbk £18, \$37.80. *An Introduction to the Electron Theory of Metals*. By G. V. Raynor. Pp.98. Pbk £10, \$21. *The Structure of Metals and Alloys*. By R. E. Smallman, W. Hume-Rothery and C. W. Haworth. Pp.407. Pbk £18, \$37.80.

Mother love

Leonard Krishtalka

Digging Dinosaurs. By John R. Horner and James Gorman. Workman Publishing, New York: 1988. Pp.210. \$17.95.

A HUNDRED years ago in the American West, a 'bone pilgrim' was a cowboy who collected dead animal bones and sent them east for processing into artificial manure. Roaming the West at the same time was a second, lesser known, breed of bone pilgrim, one who collected fossil animal bones and sent them east for processing into scientific manuscripts. Jacob Wortman, Charles Sternberg, William Reed, John Bell Hatcher and others were bone pilgrims of this sort for Pittsburgh's Carnegie Museum, New York's American Museum of Natural History, Yale's Peabody Museum and Princeton University. Today they would be called 'field palaeontologists'.

John Horner, curator of palaeontology at the Museum of the Rockies in Bozeman, Montana, would have made a fine bone pilgrim in the Old West because he is one of the finest field palaeontologists in the New West. In August 1978 Horner, then a preparator of fossils at Princeton, and his friend Bob Makela, a high-school science teacher in Montana, followed the owners of a local rock shop up a windswept knob of red and green mudstone on the plains near Choteau, Montana. Back at the rock shop lay bits of a thigh bone, a rib and a jaw of a baby hadrosaur (a duck-billed dinosaur) that the owners had found on the surface.

Horner and Makela combed the knob, and in the scree found the remains of two more baby hadrosaurs. The bones were concentrated in a bowl-shaped mass of green mudstone, a nest scooped out by the hadrosaur mother 80 million years ago. The dinosaur was a new genus, later named *Maiasaura*, 'good mother lizard'. Horner and his colleagues spent the next six years in Montana exhuming the ancient life of *Maiasaura* from the knob and surrounding rock — 14 nests, 42 eggs, three nesting grounds, 31 babies and a gigantic herd of 10,000 individuals. The excavations also uncovered the extraordinary record — 25 skeletons, 14 clutches of eggs and 19 eggs with embryonic skeletons — of a second dinosaur, a new genus of hypsilophodontid.

In a field beset by hyperbole, it is fair to say that Horner's discoveries are unparalleled. Two square miles of late Cretaceous rocks reveal geological moments when female maiasaurs and hypsilophodontids gathered in birdlike flocks on communal nesting grounds and laid their eggs. On hatching, the young maiasaurs (which were apparently altricial) stayed in their

AWFUL CHANGES MAN FOUND ONLY IN A FOSSIL STATE — REAPPEARANCE OF ICHTHYOSAURI.



A Lecture — "You will at once perceive," continued Professor Ichthyosaurus, "that the skull before us belonged to some of the lower order of animals; the teeth are very insignificant, the power of the jaws trifling, and altogether it seems wonderful how the creatures could have procured food."

The cartoon, from Francis T. Buckland's *Curiosities of Natural History* (1859), is reproduced in *Dinosaur Plots and Other Intrigues in Natural History* by Leonard Krishtalka, published by William Morrow, which will be reviewed in *Nature's Spring Books* issue next week.

nests and were fed and cared for by their good mother lizard; the hypsilophodontid hatchlings, comparatively more mature, left the nests immediately after birth. Both species, like birds, grew quickly and were warmblooded. The maiasaurs, like modern wildebeest, travelled in herds several thousand strong.

Digging Dinosaurs, which is co-written with James Gorman, is Horner's personal account of this revolutionary dinosaur diorama and the good fortune, perseverance and palaeontological detective work behind its discovery and reconstruction. Horner is folksy. He toasts the co-evolutionary ties of bonehunters and beer, using the tab of a Rainier beer can for scale in an illustration of baby maiasaur bones. Not much has changed in a hundred years of bonehunting: the field pictures are no longer black and white, the field crews no longer wear black suits and bow-ties in the quarry, and mule teams no longer haul out the dinosaur blocks. But that is about all. What has changed is the picture we have of the dinosaurs themselves, no longer cloddish and dimwitted, but hot, nimble, social and caring.

The book captures the romance, realism and scientific implications of Horner's field work and astonishing discoveries. The friendly, unassuming tone is tailored

for a general audience; those hungry for the straight, academic treatment of the evidence can refer to his papers in the scientific journals. Once in a while Horner's folksy science is, as they say in the West, bodaciously wrong — life evolved at least 3.5 not 2 billion years ago (pp. 38–39), and Darwin never called natural selection 'survival of the fittest' (p.66), Herbert Spencer did. Some critics have already demanded more evidence for maternal care among maiasaurs. Others question Horner's proposition that volcanic gassing and catastrophic flooding turned a herd of 10,000 of them into a graveyard of dismembered, shattered skeletons.

But these are the rough and tumble issues in *Digging Dinosaurs*. When it comes to dinosaurs, the palaeontologist's mind until recently was a prisoner of conventional attitudes that kept the beasts stiff and cold. As Horner says, "paleontology can grow sleepy and accumulate dust like museum cellars" were it not for challenges to the prevailing wisdom. Those challenges have wrought a dinosaur renaissance during the past two decades, a new manifesto for the Mesozoic. □

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Climate change in the circum-North Atlantic region during the last deglaciation

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A survey of new and published palaeoclimate data indicates that both the high- and low-latitude North Atlantic regions were characterized by at least three synchronous periods of abrupt climate change during the last glacial-to-interglacial transition. Climate model results suggest that changes in the melting history of the Laurentide Ice Sheet may explain much of this nonlinear response of the climate system to astronomical (Milankovitch) forcing.

ASTRONOMICAL or Milankovitch forcing has become established as the primary 'pacemaker' of the ice ages, accounting for much of the total observed climatic variance of the late Quaternary^{1,2}. Most climatic variance at frequencies lower than 1 cycle per 19,000 years is related to astronomical forcing, and even abrupt glacial terminations can be partially attributed to such forcing³. A closer look at the last glacial-interglacial transition, however, reveals a sequence of climate change that cannot be explained as a simple linear response to astronomical forcing. Marine and terrestrial records of environmental change in the high-latitude North Atlantic, Greenland and Europe reveal a period of rapid climatic warming at ~13–12.6 kyr BP, followed by an abrupt reversal towards colder glacial conditions at ~11 kyr, and a second rapid warming to interglacial conditions at ~10 kyr (refs 4–7). The abrupt 13-kyr warming and the 'Younger Dryas' cold event between 11 and 10 kyr were apparently more pronounced over Greenland and Europe than over North America, an observation that has led workers to surmise that changes in the heat budget of the high-latitude North-Atlantic produced the changes over land^{5–9}. Ruddiman⁴ proposed that a reduction in ice-sheet height over North America could have rapidly reduced the amount of cold air advected

over the North Atlantic, thus allowing the North Atlantic and downstream Europe to warm abruptly at ~13 kyr. Ruddiman⁴ and Broecker *et al.*⁵ expanded on earlier work^{10–13} to suggest that major diversions of Laurentide Ice Sheet meltwater between the Mississippi and St Lawrence rivers may have caused the North Atlantic, Greenland and Europe to cool at the beginning of the Younger Dryas period (~11 kyr) and then warm abruptly at the end of the Younger Dryas period (~10 kyr). General Circulation Model (GCM) results support the dominant role of North Atlantic sea surface temperature (SST) fluctuations in forcing high-latitude climate change^{6,9}.

Here we try to place the observed high-latitude pattern of climate change into a more global perspective. First, we examine new and published data from the Caribbean and Gulf of Mexico region that also point to systematic patterns of abrupt change at ~13–12.6, 11 and 10 kyr. We then examine new and published climate model results which suggest that much of the low-latitude pattern of change can be linked to variations in meltwater discharge down the Mississippi and St Lawrence rivers, and the sea surface temperatures of the high-latitude North Atlantic and Gulf of Mexico. Our results lead us to speculate that rapid climate events over Africa may also have been related to these same variations. We conclude with the hypothesis that the climate system translated the relatively gradual astronomical forcing, characteristic of the past 18 kyr, into an inter-related sequence of abrupt climate events over an extensive area of the globe.

Caribbean and Gulf of Mexico regions

In this section, we look at the patterns and sequence of climate change which occurred around the Caribbean and Gulf of Mexico during the last deglaciation. In particular, we focus our initial attention on data derived from an investigation of sediments from the Cariaco Basin (10°40' N, 65° W), a small (160 × 40 km), deep (1,400 m) anoxic marine basin aligned along the northern continental margin of Venezuela. This margin is

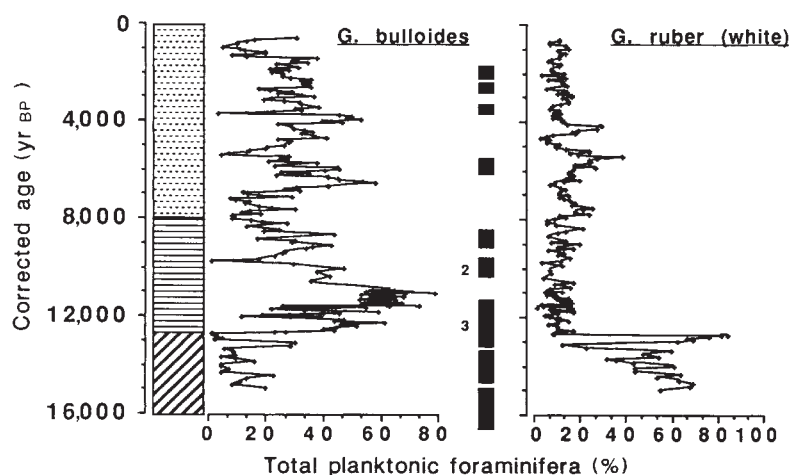


FIG. 1 Time series of *G. bulloides* and *G. ruber* relative abundance for core V12-99 from the Cariaco Basin. Also shown are core lithology and the distribution of radiocarbon-dated intervals which were biostratigraphically correlated from the closely matching, but less continuous, core V12-104. This series of dates did not include any reversals. All dates were adjusted by 400 years to correct for the difference between surface water and atmosphere radiocarbon content. The V12-104 record is less continuous due to the presence of turbidites that were not present in V12-99. ■, radiocarbon-dated intervals (2,3=number of dates); Sediment type: ▨, weakly laminated calcareous clays; ▤, strongly laminated calcareous clays; ▩, weakly bioturbated calcareous clays.

currently characterized by Ekman-induced coastal upwelling, with cold SSTs found during the dry winter/spring season of greatest along-shore wind stress, and warmer SSTs during the less windy wet summer season^{14,15}. Plankton tow data from the Cariaco Basin indicate that seasonally high abundances of phytoplankton (mainly diatoms) and the planktonic foraminifer *Globigerina bulloides* dominate the water-column assemblage during the upwelling season, whereas *Globigerinoides ruber* is abundant during the non-upwelling season¹⁶.

The strong seasonality in the wind-induced coastal upwelling is reflected in the faunal and lithological character of the laminated sediments which accumulate rapidly (20–100 cm kyr⁻¹) beneath anoxic waters in many portions of the Cariaco Basin. Piston cores V12-99 (1,005 m water depth) and V12-104 (466 m) were sampled on average every 3 and 1 cm, respectively, for a complete foraminiferal census. Age control is provided by twelve radiocarbon dates on bulk carbonate (Fig. 1). We subtracted 400 years from each date to account for the difference in radiocarbon content between the surface waters and atmosphere⁵. The two piston cores, separated by over 50 km, yielded nearly identical time series of foraminiferal abundance, indicating that our faunal records are probably representative of the basin as a whole. We focus here on the abundance record of *G. bulloides* in V12-99 as a proxy record of upwelling, and hence trade-wind intensity (Fig. 1). We will elaborate further on the very detailed Cariaco Basin record elsewhere. Like the high-latitude records of climatic change, the Cariaco Basin record shows evidence of rapid change at ~13–12.6, 11 and 10 kyr.

The presence of weakly bioturbated sediments, the high abundance of *G. ruber*, and the low abundance of *G. bulloides*

near the base of the core (Fig. 1) suggest that lowered sea level may have isolated the Cariaco Basin sufficiently to prevent active Ekman-pumping during the last glacial maximum. At 12.6 kyr, both cores record a significant increase in sedimentation rate, an abrupt change from bioturbated to distinctly laminated anoxic sediments (traceable over the entire basin), and a sudden shift in foraminiferal assemblages from a non-upwelling fauna to samples dominated by *G. bulloides*. High concentrations of *G. bulloides* during the period 12.6–10.8 kyr (>2,000 individuals g⁻¹ compared to a core mean of <500) suggest that the high sedimentation rates observed throughout this period (>80 cm kyr⁻¹ compared to the core mean of <40) were related to intense upwelling and high productivity of *G. bulloides*. The abundance of *G. bulloides* drops abruptly after 10.8 kyr, but remains at levels above the Holocene mean. This change suggests that upwelling and trade-wind intensities, although diminished, were still high during the period 10.8–10 kyr. A further decline in the abundance of *G. bulloides* after 10 kyr suggests that the Holocene mode of moderate (although still highly variable) upwelling began at this time.

The Cariaco Basin upwelling record provides evidence for rather abrupt changes in trade-wind strength at ~12.6, 10.8 and 10 kyr, with the strongest trade winds over the southern Caribbean found between 12.6 and 10.8 kyr. Independent palaeoclimate data from the region support the abrupt nature of change at these times. In the lowlands adjacent to the Caribbean, pollen and lake-level records indicate that glacial (18 kyr) aridity persisted until shortly after 11 kyr, when moist conditions suddenly prevailed^{17–19}. To the north of the Gulf of Mexico and Caribbean region, the deglacial sequence of climate change is less clear.

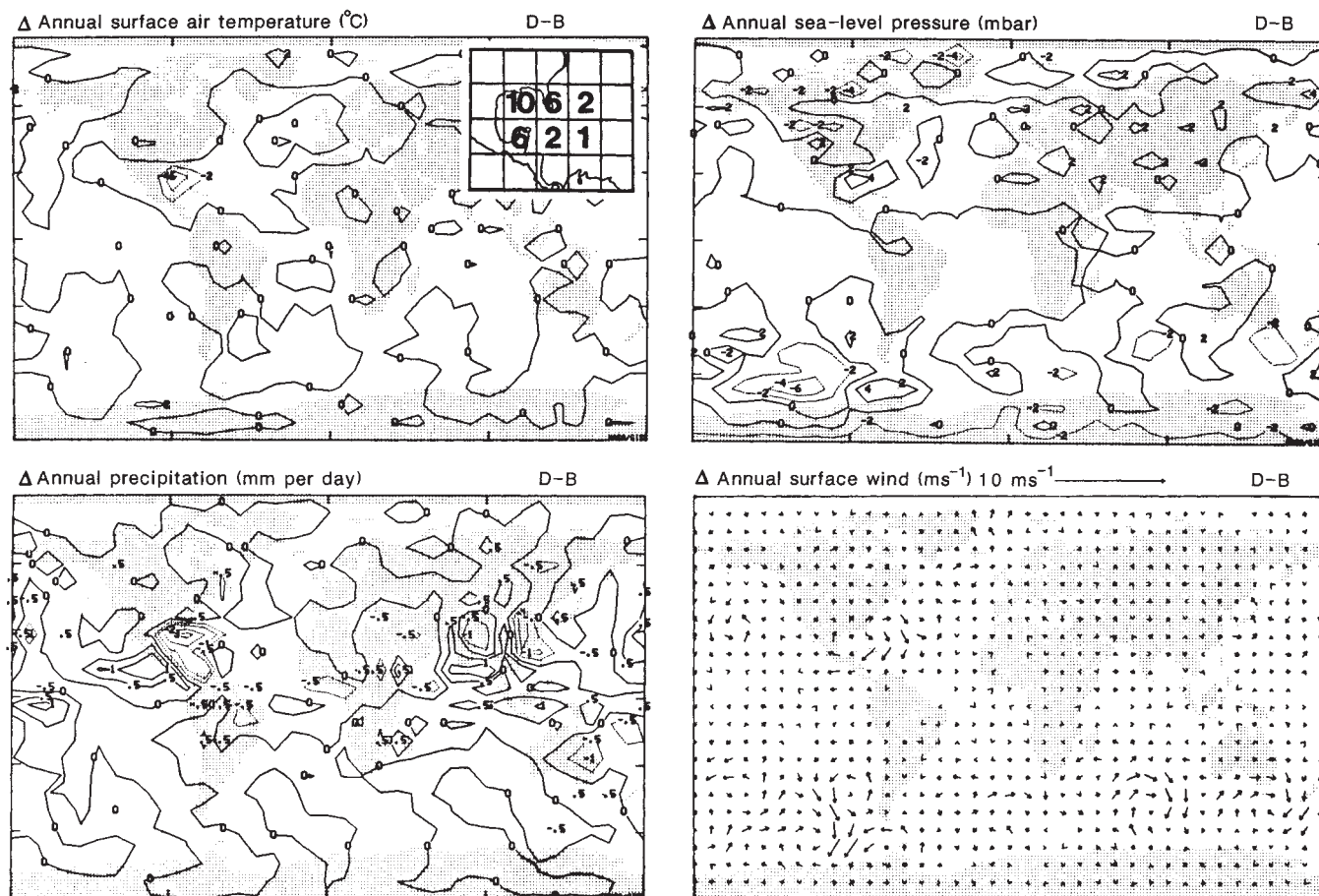


FIG. 2 Simulated mean-annual differences between experiment D and B. Inset shows the magnitude of the negative (°C) SST anomalies (relative to

the present-day) that were prescribed for six model grid points in experiment D (see Table 1 and text for more details).

Water levels in low-elevation lakes throughout the southeast US Coastal Plain were low or lakes were dry from before 18 kyr until after 15 kyr, when lake levels rose in response either to increased precipitation, rising sea level, or both²⁰. A similarly equivocal record of past hydrological change in this region is the poorly dated record of fluvial changes which seems to indicate moist conditions until 13 kyr (refs 21, 22). Pollen records from the southern Coastal Plain^{20,23-27} show abrupt change at 13 and 11 kyr. Increased amounts of herb pollen and decreased abundances of *Carya* pollen may argue for aridity between 13 and 11 kyr in Florida^{20,23,24}, just as increased amounts of *Fagus* and *Liquidambar* pollen may have signalled the onset of wetter conditions after 11 kyr further north²⁵⁻²⁷. More data are clearly required, however, to refine this regional picture.

Observed versus simulated palaeoclimatic change

The apparent synchronicity between events in the Cariaco Basin record, in other records from the Caribbean and Gulf of Mexico region, and in records from the high-latitude North Atlantic suggest a common forcing mechanism during deglaciation. In particular, the evidence for stronger trade winds between 12.6 and 10.8 kyr in the western North Atlantic and for Gulf/Caribbean aridity until ~11 kyr have led us to examine the effects that a cool Gulf of Mexico may have had on the climate system. Geological data from North America and stable isotope data from Gulf sediments suggest that the flow of cold, fresh Laurentide Ice Sheet meltwater down the Mississippi River and into the Gulf of Mexico began at ~15–14 kyr, waned slightly from ~14–13 kyr, and peaked between 13 and 11 kyr before being diverted down the St Lawrence River into the high-latitude North Atlantic^{5,9-12}. During the interval of maximum discharge into the Gulf, which coincides closely with the timing of events described above, the volume of Mississippi outflow must have been immense (5–11 times the present annual discharge, but mostly confined to the spring–summer meltwater season^{13,28}), apparently included seasonal ice-rafted detritus (R. Saucier, personal communication), and was sufficient to affect sea surface salinities well out into the western North Atlantic^{13,29}. Unlike the role of salinity, little attention has been paid to the possible consequences of meltwater-induced temperature change in the Gulf of Mexico. Independent data^{30,31} suggest that the open south-west Caribbean did not warm from glacial values until ~11 kyr, and it seems likely that the more northerly Caribbean and Gulf of Mexico regions may have been cooled even further by the large influx of glacial meltwater. We suggest that cooler Gulf SSTs during peak meltwater discharge could provide a mechanism for explaining observed events if the cooling was sufficient to produce a high regional surface-pressure anomaly, which would in turn strengthen western Atlantic trade winds and suppress precipitation.

To test this hypothesis, we compared the results from four GCM ($8^\circ \times 10^\circ$ latitude by longitude grid size) experiments (for details, see Table 1) using the Goddard Institute for Space Studies model^{6,32}. Experiment A represents our model simulation of the current (warm) climate. The results of the four-year experiments, B and C, were compared to test the sensitivity of the global climate system to the hypothesized Younger Dryas (~11–10 kyr) reduction in North Atlantic SSTs⁶. We also ran a two-year experiment (D) to test the sensitivity of the climate system to cooled Gulf of Mexico SSTs during the period ~13–11 kyr. Experiment D was identical to B, except that model grid points which included the Gulf of Mexico and adjacent water were cooled by ~6 °C on average (Fig. 2). Unfortunately, the palaeoceanographic record provides no direct evidence for cooling of the Gulf by meltwater. However, the oxygen isotope record of meltwater^{10,11,13,29} is useless in this regard because of the predominance of salinity effects, and existing microfossil-based studies of Gulf SSTs^{33,34} have sample spacings that may preclude recognition of such a brief (2-kyr) event. In the absence of data to the contrary, we selected an average cooling of 6 °C

based on estimates of meltwater volume, on the observation that present-day Mississippi River water is up to 10 °C colder than Gulf surface waters^{28,35}, and on the observation that downstream Caribbean SSTs were probably already depressed by 2–3 °C before they warmed at 11 kyr to Holocene levels^{30,31}. Independently, Oglesby *et al.*²⁸ have arrived at a similar estimate and, more importantly, have performed sensitivity tests which suggest that an even smaller cooling could still have forced the same patterns of change around the Gulf that we have forced with our SST anomaly.

Our simulated cooling of the Gulf of Mexico (D minus B) produced lower surface air temperatures, higher sea-level pressure, a large negative precipitation anomaly, and a more vigorous anticyclonic surface air circulation over the Gulf of Mexico and Caribbean region (Fig. 2). Each of these changes is statistically significant (greater than several standard deviations of the inter-annual changes found in experiment B) and agrees with the palaeoclimatic data described in the previous section. The simulated difference between experiments D and A indicate that a cool Gulf of Mexico between ~13 and 11 kyr is needed to match the palaeoclimatic evidence for greater than present aridity around the Gulf and Caribbean region, and more intense trade winds in the western North Atlantic. Averaged over the annual cycle, the cooling of the Gulf produced no significant temperature anomalies outside the Gulf/Caribbean region. In our experiment, the well-developed higher pressure and cooler temperatures in the Gulf region helped divert storm tracks northward, resulting in both lower pressure and a small band of increased precipitation north of the Gulf Coastal Plain. This pattern agrees with the observation that aridity may have been restricted to Florida and Georgia between ~13 to 11 kyr. Slightly higher precipitation values, probably indicative of increased Hadley circulation, were also simulated equatorward of the Gulf of Mexico over the northern Amazon Basin and central Andes. These changes were not as significant (less than three standard deviations) as those simulated around the Gulf of Mexico and Caribbean region, but they do appear to agree with the sparse palaeoclimate data from South America^{36,37}.

Rind *et al.*⁶ compared C and B to demonstrate the importance of high-latitude North Atlantic SSTs in forcing the observed climate changes over North America, Greenland and Europe during the last glacial-interglacial transition. Our comparisons of D with B (Fig. 2) and C with D (Fig. 3) support this conclusion. On a yearly average basis, the observed high-latitude variations in both precipitation and temperature appear to be explained by the observed changes in high-latitude SSTs: rapid warming at ~13 kyr, rapid cooling at ~11 kyr (the onset of the Younger Dryas) and rapid warming again at ~10 kyr (the end of the Younger Dryas). At lower latitudes, the warming of the Gulf at ~11 kyr can explain the wetter conditions and slight wind relaxation observed at this time in the western North Atlantic/Gulf/Caribbean region (Fig. 3). The magnitude of the

TABLE 1 Summary of General Circulation Model experiments

Designation	Description
A	current climate ³²
B	11 kyr orbital parameters ⁵⁰ 11 kyr land ice ⁴⁹ current sea surface temperatures
C	11 kyr orbital parameters ⁵⁰ 11 kyr land ice ⁴⁹ current sea surface temperatures, except in the North Atlantic north of 25°N where they were cooled to their ice age values (18 kyr) ⁵¹
D	11 kyr orbital parameters ⁵⁰ 11 kyr land ice ⁴⁹ current sea surface temperatures, except in the Gulf of Mexico region where they were cooled on average 6 °C (see Fig. 2)

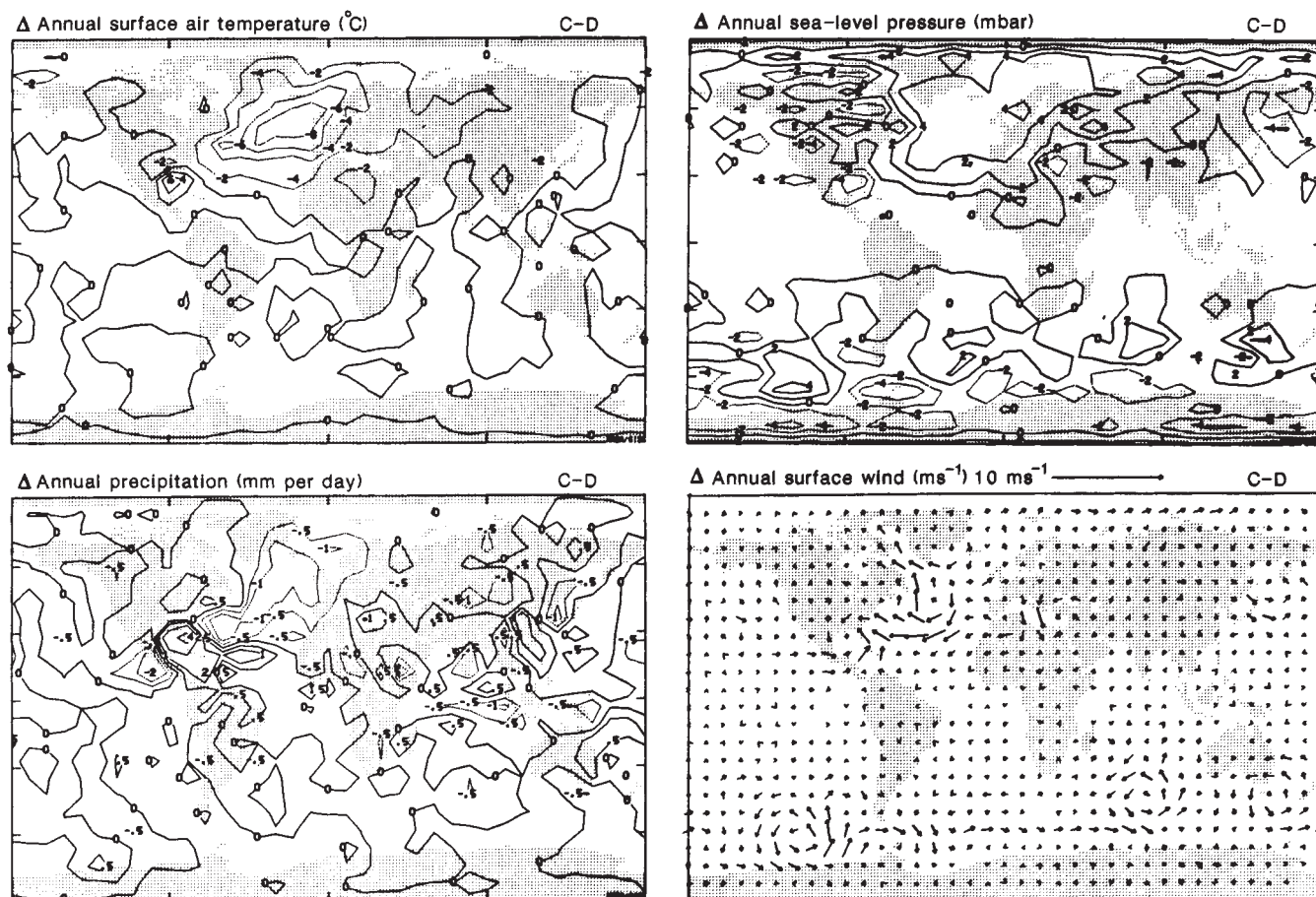


FIG. 3 Simulated mean-annual differences between experiment C and D (see Table 1 and text for more details).

Gulf/Caribbean aridity probably would have been enhanced between 13 and 11 kyr had we prescribed colder SSTs along the northern South American margin in accordance with the Cariaco Basin upwelling record.

Our simulations suggest that the observed pattern of climate change over most of the circum-North Atlantic region can be explained by the proposed effects of cold Laurentide Ice Sheet meltwater on SSTs of the high-latitude North Atlantic and Gulf of Mexico. Our model results may not, however, provide a complete physical explanation for the sequence of change observed over North Africa, and, in particular, for the arid event that apparently was synchronous with the Younger Dryas in Europe (~11–10 kyr, refs 38–46). The increased surface pressure and stronger easterly surface winds in experiment C relative to D (Fig. 3) support the idea^{39,44} that a colder North Atlantic could have stabilized the lower atmosphere and helped block the advection of moisture over North Africa. However, the simulated precipitation changes between the two experiments are somewhat ambiguous (Fig. 3). Additional experiments are needed to examine the significance of the precipitation anomalies in East and North Africa. It is also necessary to test the possibility that abrupt changes of high-latitude North Atlantic SSTs were associated with other rapid changes that could have contributed to the observed changes over North Africa. Higher atmospheric aerosols⁴⁷, changes in vegetation, a weaker monsoon, and lower-latitude Atlantic SST cooling⁴⁸ may all have helped to make North Africa more arid before ~13–12.6 kyr, and between 11 and 10 kyr.

Conclusion

Our postulated synchronicity of rapid climate events at ~13–12.6, 11 and 10 kyr around the circum-North Atlantic must be tested

by means of the acquisition of additional well-dated, high-resolution palaeoclimate records. In most cases, we were forced to use dates with an uncertainty of ~5–10% to infer synchronicity. In addition, further efforts to evaluate the palaeotemperature record from the Gulf of Mexico are clearly warranted. These palaeoclimatic studies will have to be augmented by additional climate model experiments to understand the sensitivity of the global climate system to deglacial changes in boundary conditions. Our present simulations, however, support a scenario through which the climate system (via the melting history of the Laurentide Ice Sheet) could have transformed relatively gradual and monotonic astronomical forcing into a series of abrupt, non-monotonic climate changes over a large part of the globe. □

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Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor

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Cloning and sequencing of the complementary DNA for platelet-derived endothelial cell growth factor indicates that it is a novel factor distinct from previously characterized proteins. The factor, a protein with a relative molecular mass of about 45,000, stimulates endothelial cell growth and chemotaxis *in vitro* and angiogenesis *in vivo*.

PLATELET-DERIVED endothelial cell growth factor (PD-ECGF) is an endothelial cell mitogen of relative molecular mass (M_r) ~ 45,000 (45K) purified to homogeneity from human platelets^{1,2}. By contrast with other endothelial mitogens of the fibroblast growth factor (FGF) family (reviewed in refs 3 and 4), PD-ECGF, which seems to be the only endothelial cell growth factor in human platelets², does not bind to heparin and does not stimulate the proliferation of fibroblasts¹. We describe here the protein sequencing, cDNA cloning and expression of functionally active PD-ECGF. In addition, we show that PD-ECGF has chemotactic activity for endothelial cells *in vitro* and angiogenic activity *in vivo*.

Sequence of PD-ECGF

Information about the protein sequence of PD-ECGF was obtained by the N-terminal sequencing of intact PD-ECGF, and by analysing the fragments obtained after digestion with trypsin, staphylococcal V8 protease, or CNBr (Fig. 2).

To localize a source of production of PD-ECGF, a specific

antiserum against PD-ECGF (ref. 2) was used to screen cell lines and tissues by immunoblotting. A strong 45K band was found when extract from a term placenta was analysed (data now shown). A cDNA library was therefore constructed in λ gt10 using poly(A)⁺ RNA from human placenta, and screened with oligonucleotide probes constructed using the information from the protein sequencing; the screening of 3×10^5 clones yielded three positive clones.

Nucleotide sequencing of the 1.8-kilobase (kb) insert of one of the clones (APL8) revealed a short GC-rich 5' untranslated region, an open reading frame predicting the translation of a 482-residue protein (M_r 49,972), and a short 3' untranslated sequence containing a poly(A)⁺ tail (Fig. 1). The translation is probably initiated by the ATG start codon at nucleotides 124-126, as the surrounding nucleotide sequence follows the rules for translation initiation³, whereas the sequence surrounding ATG at nucleotides 136-138 does not. Furthermore, there are no other ATG codons between an in-frame stop codon at nucleotides 28-30 and the nucleotides coding for the N-terminus of intact PD-ECGF (Fig. 2). A stop codon (TAA) is present at nucleotides 1,570-1,572, and is part of the polyadenylation signal (nucleotides 1,568-1,573). Fourteen nucleotides downstream of this signal, a long stretch of poly(A)⁺ was found. A similar overlap of the stop codon and the polyadenylation signal has been reported for human choriongonadotropin B-chain⁶.

Of the 482 amino-acids of PD-ECGF deduced from the cDNA clone, 389 were identified by amino-acid sequencing (Fig. 2). The N-terminal sequence of PD-ECGF starts 10 amino acids downstream of the proposed translation initiation site, indicating that the molecule undergoes a limited proteolytic processing after synthesis. The C-terminal amino acids predicted from the

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cDNA sequence, except the last four, were identified by amino-acid sequencing. It is not known whether PD-ECGF undergoes proteolytic processing in the C-terminus, if so, a maximum of four amino acids are removed. The M_r of the mature protein would thus be 48.6–49K, in reasonable agreement with the estimate of 45K obtained from SDS-gel electrophoresis of pure PD-ECGF^{1,2}.

The predicted amino-acid sequence from the cDNA clone matched the previously obtained amino-acid sequence except

at position 471, where the nucleotide sequence predicts Leu, whereas Ser was found in both of two different peptides obtained from this region (Fig. 2). It is possible that polymorphism occurs at this position.

Matching the sequence of PD-ECGF with those of the PIR, EMBL and Genbank databases (releases 17, 14 and 56, respectively) revealed no striking similarities to other proteins. Short internal repeats in the sequence were noted (overlined in Fig. 2). One important feature of the sequence is the lack of a

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1 GGGCAGTGGG CCGCTGTGGG CGAACCTGGA ACCCTACGGT CCGGACCCGC GGGGAGGGCC
61 GGGTACTCTGG GCTGGGATCC GGAGCAGGCG GGGGAGGGCA GGGCCCTAAG CAGGCCCGGA
121 GCG ATG GCA GCC TTG ATG ACC CCG GGA ACC GGG GGC CCA CCC GCG CCG GGT GAC TTC TCC
1 Met Ala Ala Leu Met Thr Pro Gly Thr Gly Ala Pro Pro Ala Pro Gly Asp Phe Ser
181 GCG CAA GCG AGC CAG GGA CTT CCC GAG CCG TCG CCA GAG CCC AAG CAG CTC CCG GAG CTG
20 Gly Glu Gly Ser Gln Gly Leu Pro Asp Pro Ser Pro Glu Pro Lys Gln Leu Pro Glu Leu
241 ATC CGC ATG AAG CGA GAC GGA GGC CGC CTG AGC GAA GCG GAC ATC AGG GGC TTC GTG GCC
40 Ile Arg Met Lys Arg Asp Gly Arg Leu Ser Glu Ala Asp Ile Arg Gly Phe Val Ala
301 CGT CTG GTG AAT GGG AGC CCG CAG GCG CCA CAG ATC GCG GCG ATG CTG ATG GGC ATC CGA
60 Ala Val Val Asn Gly Ser Ala Gln Gly Ala Gln Ile Gly Ala Met Leu Met Ala Ile Arg
361 CTT CGG GGC ATG GAT CTG GAG GAG ACC TCG GTG CTG ACC CAG GCC CTG GCT CAG TCG GGA
80 Leu Arg Gly Met Asp Leu Glu Glu Thr Ser Val Leu Thr Gln Ala Leu Ala Gln Ser Gly
421 CAG CAG CTG GAG TGG CCA GAG GCC TGG CGC CAG CAG CTT CTG GAC AAG CAT TCC ACA GGG
100 Gln Gln Leu Glu Trp Pro Glu Ala Trp Arg Gln Gln Leu Val Asp Lys His Ser Thr Gly
481 GGT GTG GGT GAC AAG GTC AGC CTG CTC GCA CCT GCC CTG GCG GCA TGT GGC TGC AAG
120 Gly Val Gly Asp Lys Val Ser Leu Val Leu Ala Pro Ala Leu Ala Ala Cys Gly Cys Lys
541 GTG CCA ATG ATC AGC GGA CCG GGT CTG GCG CAC ACA GGA GCG ACC TTG GAT AAG CTG GAG
140 Val Pro Met Ile Ser Gly Arg Gly Gly His Thr Gly Thr Gly Thr Leu Leu Leu Leu
601 TCT ATT CCT GGA TTC AAT GTC ATC CAG AGC CCA GAG CAG ATG CAA GTG CTG CTG GAC CAG
160 Ser Ile Pro Gly Phe Asn Val Ile Gln Ser Pro Glu Gln Met Gln Val Leu Leu Asp Gln
661 GCG GGC TGC TGT ATC CTG GGT CAG AGT GAG CAG CTG GTC CTT GCG CAG GGA ATC CTA TAT
180 Ala Gly Cys Cys Ile Val Gly Gln Ser Glu Gln Leu Val Pro Ala Asp Gly Ile Leu Tyr
721 GCA GCC AGA GAT GTG ACA GCC ACC CTG GAC AGC CTG CCA CTC ATC ACA GCC TCC ATT CTC
200 Ala Ala Arg Asp Val Thr Ala Thr Val Asp Ser Leu Pro Leu Ile Thr Ala Ser Ile Leu
781 AGT AAG AAA CTC GTG GAG GGG CTG TCC GGT CTG GTG GTG GAC CTT AAG TTC GGA GGG GCC
220 Ser Lys Lys Leu Val Glu Gly Leu Ser Ala Leu Val Val Asp Val Lys Phe Gly Gly Ala
841 GCG CTC TTC CCC AAC CAG CAG CAG CCG GCG GAG CTG CCA AAG ACC CTG GTT GGC GTG GGA
240 Ala Val Phe Pro Asn Gln Glu Gln Ala Arg Glu Leu Ala Lys Thr Leu Val Gly Val Gly
901 GCG AGC CTA GGG CTT CCG GTC GCG GGA GCG CTG ACC GCC ATG GAC AAG CCC CTG GGT CCG
260 Ala Ser Leu Gly Leu Arg Val Ala Ala Ala Leu Thr Ala Met Asp Lys Arg Leu Gly Arg
961 TGC GTG GCG CAG GCG CTG GAG CTG GAG GAG GCG CTG CTC TGC ATG GAG GCG GCA GCG CCG
280 Cys Val Gly His Ala Leu Glu Val Glu Glu Ala Leu Leu Cys Met Asp Gly Ala Gly Pro
1021 CCA GAC TTA AGG GAC CTC GTC ACC AGC CTC GGG GCG GCC CTG CTC TGG CTC AGC GGA CAC
300 Pro Asp Leu Arg Asp Leu Val Thr Thr Leu Gly Gly Ala Leu Leu Trp Leu Ser Gly His
1081 GCG GCG ACT CAG GCG CAG GCG GCT GCG GCG GCG GCG GCG CTG GAC GAG GCG TCG GCC
320 Ala Gly Thr Gln Ala Gln Gly Ala Ala Arg Val Ala Ala Leu Asp Asp GAG GCG TCG GCC
1141 CTT GCG CCG TTC GAG CCG ATG CTG GCG GCG CAG GCG GTG GAT CCC GGT CTG GCG CCA GCG
340 Leu Gly Arg Phe Glu Arg Met Leu Ala Ala Glu Val Asp Pro Gly Leu Ala Arg Ala
1201 CTG TGC TCG GGA AGT CCC GGA CCA GCG CCG CAG CTG CTG CCG GCG CCG GAG CAG GAG
360 Leu Cys Ser Gly Ser Pro Glu Ala Glu Arg Gln Leu Pro Arg Ala Arg Glu Gln Glu
1261 GAG CTG CTG GCG CCG CCA GAT GCG ACC GTG GAG CTG CTC CCG GCG CTG CCG CTG CCG CTG
380 Glu Leu Leu Ala Pro Ala Asp Gly Thr Val Glu Leu Val Arg Ala Leu Pro Leu Ala Leu

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1321 GTG CTG CAC GAG CTC GGG GCC GGG CCG AGC CCG GCT GGG GAG CCG CTC CCG CTC GGG GTG
400 Val Leu His Glu Leu Gly Ala Gly Arg Ser Arg Ala Gly Glu Pro Leu Arg Leu Gly Val
1381 GCG CCA GAG CTG CTG GTC GAC GTG GGT CAG AGG CTG CCG CGT GGG ACC CCC TGG CTC CGC
420 Gly Ala Glu Leu Leu Val Asp Val Gly Gln Arg Leu Arg Arg Gly Thr Pro Trp Leu Arg
1441 CTG CAC CCG GAG GCG CCC GCG CTC AGC GCG CCG CAG AGC CCG CCG CTG CAG GAG GCG CTC
440 Val His Arg Asp Gly Pro Ala Leu Ser Gly Pro Gln Ser Arg Ala Leu Gln Glu Ala Leu
1501 GTA CTC TCC GAC CCG GCG CCA TTC GCC CCG CCC TTG CCC TTC GCA GAG CTC GTT CTG CCG
460 Val Leu Ser Asp Arg Ala Pro Phe Ala Ala Pro Leu Pro Phe Ala Glu Leu Val Leu Pro
1561 CCG CAG CAA TAA AGC TCC TTT GCG GCG AAA (A)n
480 Pro Gln Gln *

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FIG. 1 Nucleotide sequence of PD-ECGF. The putative initiation codon is at nucleotides (nt) 124–126. An in-frame stop codon (nt 28–30) upstream of the initiation codon is marked with dots and a stop codon at nt 1,570–1,572 with an asterisk. The polyadenylation signal (nt 1,568–1,573) is underlined with an arrow. Regions corresponding to the prepared oligonucleotide probes 228, 231, 240, 258 and 259, are underlined.

METHODS. Total RNA was isolated from a human placenta of 22-gestational-weeks by the method of Han *et al.*²¹. A cDNA library was constructed in λ gt10 from the poly(A)⁺ RNA following the method described by Watson *et al.*²², with some modifications: following the first strand synthesis, oligo(dG) was added to its 3' end by terminal transferase; second strand synthesis was primed by addition of an external primer of oligo(dC), as well as internal RNA primers made by RNaseH. Five unique oligonucleotides were deduced from the amino-acid sequence by the method of Lathe²³ and prepared by an Applied Biosystems DNA synthesizer 381A. The oligonucleotide probes were labelled by end-tailing or primer extension. Independent clones (~300,000) from the human placenta λ gt10 cDNA library were screened with these probes separately following the method described by Ullrich *et al.*²⁴. Three clones were found to be positive both with probes 228 and 240. The cDNA inserts were shown to be colinear by restriction mapping. The cDNA insert of λ PL8, with the longest insert of ~1.8 kb, was subcloned into M13mp18 and Bluescript (Stratagene). Deletion mutants were made by the sequential deletion method of Yanisch-Perron *et al.*²⁵. Both strands of the cDNA were sequenced by the dideoxy-termination method²⁶. Sequences that were difficult to determine by this procedure were examined by the Maxam-Gilbert method²⁷.

FIG. 2 Amino-acid sequence of PD-ECGF (one-letter code). N-terminal sequence (—), tryptic fragments (· · ·), staphylococcal protease V8 fragments (++) and a CNBr fragment (==) of PD-ECGF are indicated, as well as Cys residues (*), a potential N-glycosylation site (■), and a possible site of polymorphism (▲). Internal repeats are overlined.

METHODS. To prepare tryptic fragments, 40 μ g pure PD-ECGF² was desalted on a C4 narrow-bore reversed-phase column (Brownlee Aquapore BU-300; 2.1 $\times$ 300 mm), eluted with 0.1% trifluoroacetic acid and a gradient of acetonitrile, and then dried in a Speedvac Concentrator and redissolved in 200 μ l 6 M guanidine-HCl, 0.25 M Tris-HCl, pH 8.5, 1 mM EDTA, containing 100 μ g dithiothreitol. The solution was flushed with N₂ for 20 s and left at room temperature for 3 h, at which time 2 μ l 4-vinyl-pyridine was added. After a further 3 h at room temperature, the sample was desalted by chromatography on the C4 column. The volatile solvent was removed as above and PD-ECGF was digested with TPCK-trypsin (Sigma; substrate/enzyme ratio, 50 (w/w)) in 0.1 M ammonium bicarbonate, containing 2 M urea, for 4 h at 37°C. The tryptic fragments were immediately loaded onto a C4 narrow-bore, reversed-phase HPLC column eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Non-homogeneous peptides were re-run on HPLC narrow-bore reversed-phase column under different conditions. The column temperature was kept at 35°C, the flow rate was 100 μ l min⁻¹ and the effluents were monitored at 220 nm. Fractions were collected manually in polypropylene tubes. Peptides were also isolated from PD-ECGF fragmented with staphylococcal V8 protease or CNBr

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MAALMTPTGTGAPPAGDFSGEGSGOGLPDPSPEPKQLPELIRMKRDGRLSEADIRGFVAA 60
/-----/-----/-----/-----/-----/-----/
VVGSAQAQAIGAMLMAIRLRGMDELETSVLTOALAQSGOQLEWPEAWROQLVDKHSHTGG 120
/-----/-----/-----/-----/-----/-----/
VGDVSLVLAPALAACGCKVPMISGRGLGHTGTLTKLESIPGFNVISPEQMOVLDDQA 180
/-----/-----/-----/-----/-----/-----/
GCCIVGQSEQLVPADGILYAARDVTATVDSLPLITASILSKKLVEGLSALVVDVFKGGAA 240
/-----/-----/-----/-----/-----/-----/
VFPNQEQARELAKTLVGVGASLGRVAAAL TAMDKPLGRVGHALVEVEALLCMQDAGPP 300
/-----/-----/-----/-----/-----/-----/
DLRDLVTTLGGALLWLSGHAGTQAQGAARVAAALDDGSALGRFERMLAAQGVDPGLARAL 360
/-----/-----/-----/-----/-----/-----/
CSGSPAERRQLLPRAREQEELLAPADGTVELVRALLPLALVHELHAGRSRAGEPLRLGVG 420
/-----/-----/-----/-----/-----/-----/
AELLVDVGORLRRGTPLWRVHRDGPALSGPOSRAALQFALVSDRAPFAAPLFAELVLPQQ 482
/-----/-----/-----/-----/-----/-----/

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by similar methods. The amino-acid sequences of the peptides were determined by use of an automated gas-phase sequencer (Applied Biosystems Protein sequencer, model 470A, with an online PTH-analyser, model 120A).

hydrophobic signal sequence, suggesting that PD-ECGF is not a classical secretory protein. The sequence contains only one Tyr, which is probably not exposed on the surface of the molecule, as attempts to radiolabel PD-ECGF with ^{125}I using the chloramine-T method resulted in a very low incorporation of radioactivity². There is one potential *N*-glycosylation site, Asn 63. The PTH-amino-acid yield of the corresponding tryptic peptide decreased dramatically, just before this residue (Fig. 2). This could be due to the formation of a cyclic compound of the Asn residue and the following Gly residue⁷, which can occur only if the Asn residue is non-glycosylated. This, in combination with the M_r of PD-ECGF as determined by SDS-gel electrophoresis, compared with the M_r predicted from the cDNA clone, indicates that Asn 63 is not glycosylated. There are a total of seven Cys residues, indicating that the molecule contains at least one free thiol group.

Southern and northern blotting

To obtain information about the structure of the human PD-ECGF gene, Southern blotting analysis of human DNA was performed. Hybridization with a PD-ECGF cDNA probe revealed single bands of 19 kb and 9.2 kb after digestion with *Hind*III and *Xba*I, respectively (Fig. 3a). This indicates that only one copy of the PD-ECGF gene is present in the human genome. Indeed, analysis of human genomic PD-ECGF clones supports this conclusion (K.H. *et al.*, manuscript in preparation). Human placenta poly(A)⁺ RNA was examined by northern blotting; a single transcript of about 1.8 kb was recognized by the PD-ECGF cDNA probe (Fig. 3b). As the longest cDNA clone found, λ PL8, has an insert of about 1.8 kb, it probably represents a full-length copy of the PD-ECGF transcript.

Expression of PD-ECGF cDNA

To verify the authenticity of the cDNA clone, it was expressed in NIH 3T3 cells. The λ PL8 insert was subcloned into the pLJ expression vector to give pLPL8J. This vector has Molony leukaemia virus long terminal repeat as a promoter to drive the cDNA transcription, as well as a neomycin-resistance gene as a selection marker⁸. pLPL8J and pLJ were introduced into the NIH 3T3 cells by the calcium phosphate co-precipitation method, and cell lines were selected by neomycin. Analysis of one cell line transfected with pLPL8J revealed growth-promoting activity for porcine aortic endothelial cells in the cell lysate, but not in the conditioned medium (Fig. 4a,b). The activity was completely neutralized by a specific rabbit antiserum against PD-ECGF, indicating that the activity was due to the synthesis of functionally active PD-ECGF by the cell line (Fig. 4a). The NIH 3T3 cell line transfected with only the pLJ vector did not contain any growth-promoting activity for porcine aortic endothelial cells. Furthermore, the cell lysate of the cell line transfected with pLPL8J was found to contain a 45K component in immunoblotting experiments in which the antiserum against PD-ECGF was used (Fig. 4c). The size of the product was similar to that of human PD-ECGF purified from platelets (Fig. 4c). These data show that the purified, sequenced and cloned PD-ECGF molecule is responsible for the observed biological effect on porcine endothelial cells. That PD-ECGF produced by the transfected NIH 3T3 cells remained inside the producer cells and was not secreted to any appreciable extent into the cell culture medium, is consistent with the lack of a signal sequence in the protein (Fig. 1). This is a property that PD-ECGF shares with acidic FGF (ref. 9) and basic FGF (ref. 10). It is not known whether there are alternative mechanisms to release these factors from their producer cells.

Chemotactic activity of PD-ECGF

PD-ECGF was found to have a chemotactic effect on bovine aortic endothelial cells, with half-maximal activity at about 1 ng ml^{-1} (Fig. 5a). Consistent with its mitogenic target cell specificity for endothelial cells, PD-ECGF did not induce

smooth muscle cell migration. Controls using serum- and platelet-derived growth factor induced extensive smooth-muscle-cell migration in the same experiment. Checkerboard analysis revealed that endothelial cell migration induced by PD-ECGF was due to directed cell migration (chemotaxis) and not to random migration (chemokinesis) (Fig. 5b). The chemotactic activity of PD-ECGF was neutralized by PD-ECGF antibodies (Fig. 5c).

Angiogenic activity of PD-ECGF

Two different approaches were taken to investigate whether PD-ECGF has angiogenic activity *in vivo*. First, we investigated the effect of PD-ECGF on the developing vascular system of the chick chorioallantoic membrane. Partially purified PD-ECGF consistently induced a strong angiogenic response (Fig. 6a), which was inhibited by antibodies against PD-ECGF (Fig. 6b). Thus, the angiogenic response is unlikely to be due to inflammation or other factors than PD-ECGF present in the preparation. Furthermore, pure PD-ECGF, at a dose of 50 ng, induced angiogenesis in the chorioallantoic membrane (data not shown).

Second, we investigated the effect of transfection of PD-ECGF cDNA into tumour cells on the vascularization of tumours formed by these cells in nude mice. NIH 3T3 cells transformed by an activated human Ha-ras gene¹¹ (termed a1-1 cells) were chosen as target cells and were transfected with a plasmid containing PD-ECGF DNA (pLPL8J), or with a control plasmid without insert (pLJ). After transfection, cells were selected by G418 resistance, and then injected subcutaneously into nude mice. After 2 weeks, tumours had grown to ~2 cm in diameter from both cell types; they were then collected and fixed in formaldehyde. A histological examination revealed that the tumours that developed from a1-1 cells, transfected with

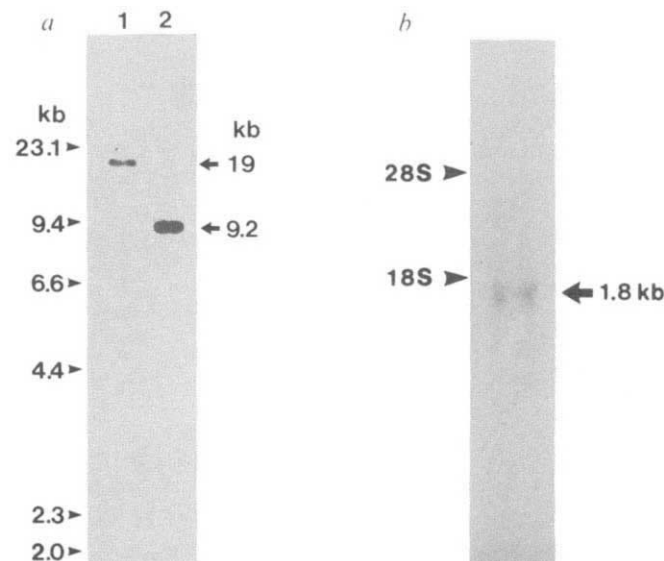


FIG. 3 a, Southern blot analysis of *Hind*III (lane 1) or *Xba*I (lane 2) digests of human DNA. b, Northern blot analysis of poly(A)⁺ RNA from human placenta. Migration positions of ribosomal RNA markers are shown on the left. Blots were hybridized with PD-ECGF cDNA probes.

METHODS. High-molecular weight human genomic DNA was prepared from normal human leukocytes. Samples of $10 \mu\text{g}$ each were digested by restriction enzymes, subjected to electrophoresis in agar and blotted on to nitrocellulose membranes (Schleicher and Schüll). Poly(A)⁺ RNA ($1 \mu\text{g}$), purified from human placenta of 22 weeks' gestation as described in the legend to Fig. 1, was electrophoresed in a 0.9% formalin-denaturing gel and blotted on to Hybond-N nylon filter (Amersham). Hybridization was performed in a solution of 50% formamide, 0.65 M NaCl, 0.1 M sodium PIPES, pH 6.8, 10% dextran sulphate, $5 \times$ Denhardt's solution, 0.1% SDS, 4 mM EDTA and $100 \mu\text{g ml}^{-1}$ salmon sperm DNA at 42°C for 18 h, and then washed four times with $2 \times \text{SSC}$, 0.2% sodium phosphate, 0.1% SDS for 20 min periods at 50°C .

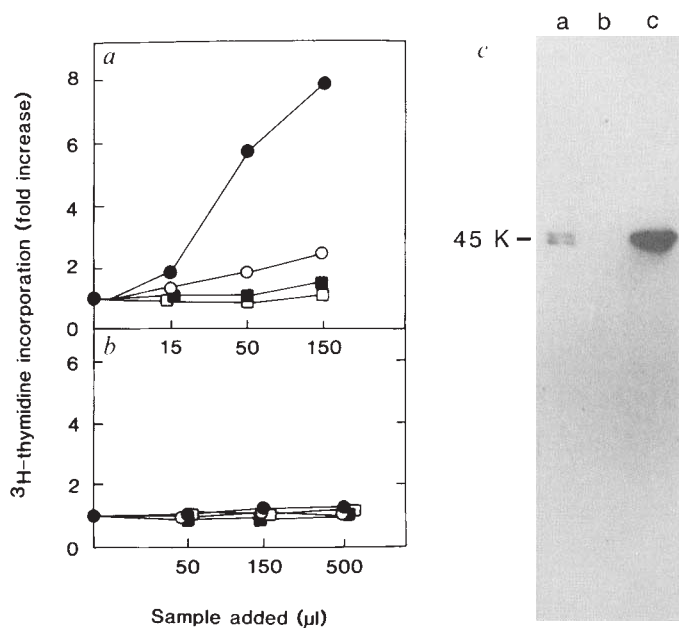
pLPL8J, contained a very high density of blood vessels (Fig. 6c), by contrast with tumours from a1-1 cells transfected with pLJ without cDNA insert, which contained only a few blood vessels (Fig. 6d). In conclusion, PD-ECGF has a potent angiogenic activity both in the chorioallantoic membrane assay and when expressed by tumour cells growing in nude mice.

Discussion

Angiogenesis is an important process during, for example, embryogenesis, wound healing and organ regeneration. In addition, aberrant angiogenesis occurs in several pathological conditions, such as in tumour growth, in certain retinopathies, and in rheumatoid arthritis. Angiogenesis is a complex process that involves several steps, including migration and proliferation of endothelial cells¹². The best characterized of the factors that

have been found to stimulate angiogenesis are FGFs, a family of heparin-binding endothelial cell mitogens that were originally purified from neural tissues^{3,4}. Additionally, transforming growth factor (TGF)- α ¹³, TGF- β ^{14,15}, tumour necrosis factor- α ^{16,17} and angiogenin¹⁸ have been found to have angiogenic activity. PD-ECGF, acidic and basic FGFs, and TGF- α are the only factors that have been shown to stimulate endothelial cells both *in vitro* and *in vivo*^{3,4,13}. The target cell specificities of these factors, however, differ: FGFs and TGF- α stimulate proliferation of a wide variety of cells including fibroblasts, whereas PD-ECGF so far has not been found to stimulate any cell type other than endothelial cells. Furthermore, the deduced primary structure of PD-ECGF shows no similarity with other known proteins. Thus, the biological and structural properties of PD-ECGF indicate that it is a novel type of angiogenic factor that

FIG. 4 Biosynthesis of PD-ECGF in NIH 3T3 cells transfected with pLPL8J. Growth factor activity from a cell lysate (a) and the conditioned medium (b) of NIH 3T3 cells, transfected with PD-ECGF cDNA (NIH3T3-pLPL8J; circles) or with the corresponding vector without insert (NIH3T3-pLJ; squares) in the absence (filled-in symbols) and presence (open symbols) of PD-ECGF antibodies². PD-ECGF protein was also visualized by immunoblotting using PD-ECGF antiserum (c); lane a, PD-ECGF purified from human platelets; lane b, cell lysate for NIH 3T3-pLJ cells; lane c, cell lysate from NIH3T3-pLPL8J cells. METHODS. NIH 3T3 cells transfected with pLJ or with pLPL8J were grown to confluence in 10-cm cell-culture dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and antibiotics. The cells were then cultured for 24 h in serum-free conditions in DMEM supplemented with 1% bovine serum albumin, 7.8 $\mu\text{g ml}^{-1}$ cholesterol, 5.5 $\mu\text{g ml}^{-1}$ oleic acid, 8 $\mu\text{g ml}^{-1}$ L- α -phosphatidylcholine and 0.2 mg ml^{-1} transferrin. After collecting the conditioned media, cells were washed twice in PBS and then scraped into 1 ml PBS. Following careful resuspension, a cell lysate was prepared by disrupting the cells by three cycles of freezing and thawing, followed by centrifugation at 25,000g for 30 min and collection of the supernatants. Growth-promoting activity was measured by the incorporation of ^3H -thymidine into porcine aortic endothelial cells¹ in the absence or presence of 2 $\mu\text{g ml}^{-1}$ PD-ECGF antibody purified by protein A-Sepharose². For immunoblotting, cell lysates were prepared by washing and resuspending the cells in PBS containing 1 mM phenylmethylsulphonyl fluoride (Sigma), 150 KIU aprotinin (Sigma) and 10 mM EDTA. The lysates and 100 ng pure PD-ECGF² were subjected to SDS-gel electrophoresis in 10–18% gradient polyacrylamide gel under reducing conditions²⁸. After transfer to a nitrocellulose membrane, immunoblotting was performed as described²⁹, using a 1:50 dilution of PD-ECGF antiserum². ^{125}I -labelled staphylococcal protein A, bound to antibodies on the blot, was visualized by autoradiography. PD-ECGF



purified from platelets occurs as a doublet of about 45K, probably as a result of proteolysis during preparation².

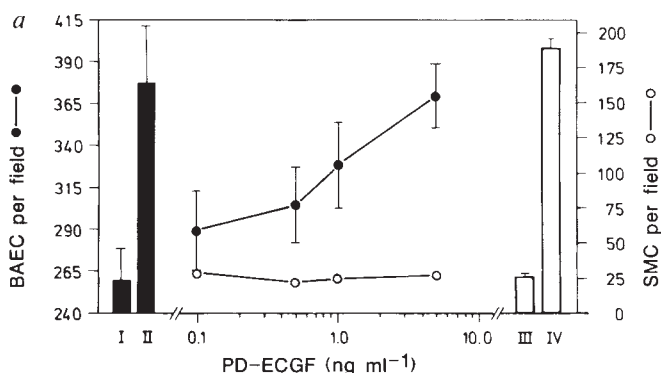
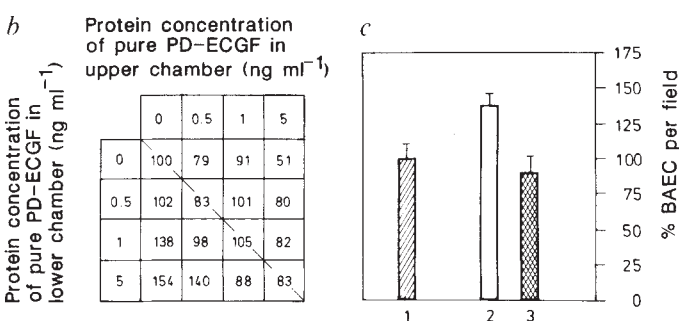


FIG. 5 Chemotactic activity of PD-ECGF. a, The migration of bovine aortic endothelial cells (BAEC; closed circles and left ordinate) and smooth muscle cells (SMC; open circles and right ordinate) towards different concentrations of PD-ECGF. Controls are shown as a histogram: I, BAEC medium only; II, BAEC medium plus 10 ng ml^{-1} basic FGF; III, SMC medium only; IV, SMC medium plus 5% FCS. b, Checkerboard analysis of BAEC migration. The data represent cell number per field (%). Standard deviations were below 15%. The values below the diagonal indicate chemotaxis. c, The chemotactic activity of PD-ECGF is neutralized by PD-ECGF antibodies: 1, buffer control; 2, pure PD-ECGF (5 ng ml^{-1}); 3, pure PD-ECGF (5 ng ml^{-1}) and anti-PD-ECGF



antibodies (300 ng ml^{-1}) (purified by protein A-Sepharose).

METHODS. Chemotaxis assays of BAEC and SMC were performed in 48-well microchambers as described³⁰. Gelatin and fibronectin coating of the Nucleopore filters was necessary for BAEC adhesion. Cells (18,000 BAEC or 25,000 SMC per well) were added to the upper wells. Serum-free DMEM was used for SMC migration, and DMEM supplemented with 1% FCS for BAEC migration. The number of cells that had migrated during a 5 h incubation period to the lower surface of the filter were counted in three high-power fields. All experiments were done in triplicate.

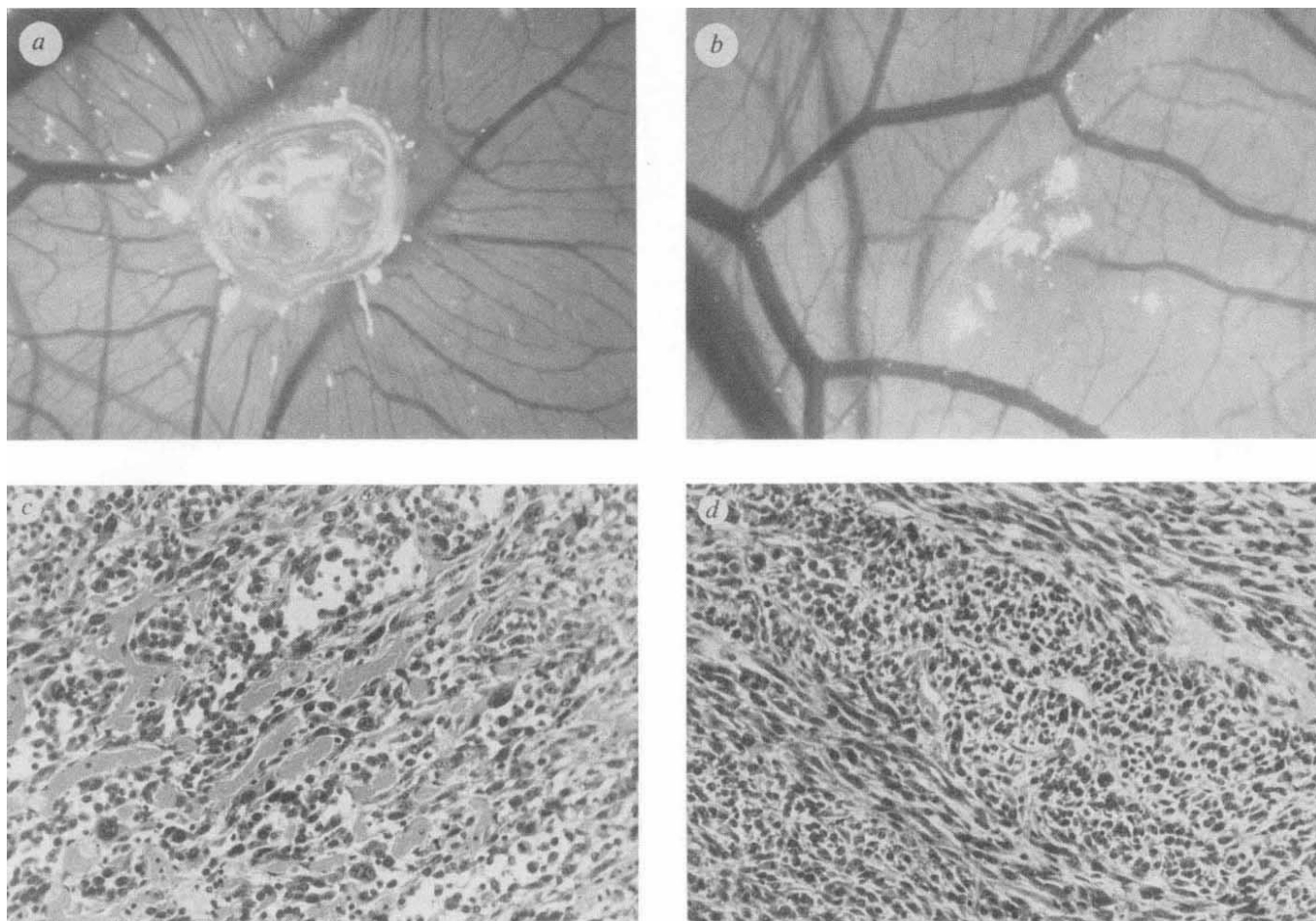


FIG. 6 Angiogenic properties of PD-ECGF. *a*, and *b*, The induction of angiogenesis in the chick chorioallantoic membrane. *a*, Partially purified PD-ECGF (purified through the hydroxylapatite step², 1% pure, 1.2 mg of protein ml⁻¹; 5 μ l per methylcellulose disk) induced angiogenesis on the chick chorioallantoic membrane (89% positive results, n (number of embryos)=65). *b*, Partially purified PD-ECGF incubated for 20 min with anti-PD-ECGF antibodies (2.5 μ l each solution) (59% negative results, n =22). In addition, 50 ng pure PD-ECGF induced angiogenesis (93% positive results, n =15). Test substances were incorporated into methylcellulose disks (10 μ l) and transplanted onto 9-day-old chorioallantoic membranes as described^{31,32}. The assays were analysed daily for 2 days. Disks were visible by their light reflections. *c* and *d*, The angiogenic effect of PD-ECGF after

expression by tumour cells growing in nude mice. a1-1 cells were transfected with pLJ (control) or pLPL8J (carrying a PD-ECGF insert) by electroporation (Biorad, Gene Pulser). Transfected cell clones were selected by G418 resistance. All a1-1 clones transfected with pLPL8J were found to have growth-promoting activity on porcine aortic endothelial cells, which could be inhibited by PD-ECGF antibodies (data not shown); a1-1 cells transfected with pLJ had no such activity. a1-1 cells transfected with pLPL8J (*c*) and cells transfected with pLJ (*d*) were injected subcutaneously at a cell number of 1×10^5 into nude mice and tumours of ~2-cm diameter developed in ~2 weeks. Tumours were collected, fixed with formaldehyde, stained with hematoxylin-eosin and examined with light-microscope. The photographs represent an 825-fold magnification.

may act specifically on endothelial cells. The finding that PD-ECGF is present in the placenta is intriguing, since angiogenesis is an important process in the development of this organ.

The restricted target cell specificity of PD-ECGF and the fact that platelets are a main source of it, suggest that PD-ECGF has a role in maintaining the integrity of the blood vessels. After injury to the endothelial cell layer of vessels, platelets aggregate at the subendothelial space. Platelet-release products, such as platelet-derived growth factor, are thought to be involved in the development of atherosclerotic lesions by stimulating migration and proliferation of smooth muscle cells and fibroblasts in the arterial media^{19,20}. Although the precise mechanism of secretion of PD-ECGF is not known at present, it is possible that PD-ECGF counteracts the atherogenic effect of other platelet products by promoting repair of the endothelial cell layer. The availability of functionally active recombinant PD-ECGF (Fig. 4), combined with a high yield purification method², cDNA clones (Fig. 1) and specific antibodies², now makes it possible to address questions related to the function of PD-ECGF *in vivo*. □

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LETTERS TO NATURE

A marked concentration of galaxy clusters: is this the origin of large-scale motions?

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THE distances and velocities of 400 elliptical galaxies¹, out to redshifts equivalent to recession velocities of $\sim 6,000 \text{ km s}^{-1}$, suggest that the peculiar velocities obtained by subtraction of the general cosmological expansion are best fitted by a flow induced by a 'great attractor'², a large mass of $5.4 \times 10^{16} M_{\odot}$ and centred on galactic longitude $l = 307^{\circ}$ and latitude $b = 9^{\circ}$ at a distance $R_m = 4,350 \pm 350 \text{ km s}^{-1}$. A redshift survey of ~ 900 galaxies³ shows that the excess galaxy number counts in this direction are due to two substantial concentrations of galaxies at recession velocities $v \approx 3,000 \text{ km s}^{-1}$ and $4,500 \text{ km s}^{-1}$. Here we show that in roughly the same direction there is also a very rich concentration of galaxy clusters which may have a considerable dynamical influence. The estimated redshift distances of these clusters range from $3,000$ to $20,000 \text{ km s}^{-1}$, with a main complex at $v \approx 14,000 \text{ km s}^{-1}$. The barycentre of this concentration lies $\sim 25^{\circ}$ away from the cosmic microwave background dipole⁴, and $\sim 10^{\circ}$ away from the latest reported position of the Great Attractor^{5,6}.

The distribution of clusters of galaxies is of fundamental significance in understanding the matter distribution, in explaining the origin of the observed peculiar velocity field^{7,2}, and in estimating both the local peculiar acceleration vector and the distance at which the Hubble flow becomes unperturbed⁸. Although their number is relatively small, the clusters, being peaks of the density field, might avoid the problems that some other all-sky density tracers (for example, IRAS galaxies^{9–12}) have in describing the large-scale density field.

We have analysed the southern part of the new Catalogue of Rich Clusters of Galaxies¹³ (hereafter ACO), which lists 1,638 clusters with declination $\delta \leq -17^{\circ}$. This catalogue lists redshifts for 145 clusters. The incompleteness of the sample with respect to redshift means that, contrary to what has been done in the Northern Hemisphere¹⁴, it is not sufficient to use those few clusters with known velocity to map the large-scale distribution of clusters in the Southern Hemisphere. We have therefore used 143 redshifts (z) to estimate a linear relation between $\log [D_L(z)] + 0.2K(z)$ and the magnitude of the tenth brightest member, including also a correction for the cluster richness (Scott effect). Here, $D_L(z)$ is the luminosity distance, assuming cosmological deceleration $q_0 = 1/2$, and $K(z)$ is the K-correction for E and S0 galaxies¹⁵. We find that the relation has a dispersion

$\sigma_{\text{sample}} \approx 0.11$ in $\log [D_L(z)] + 0.2K(z)$, corresponding to $\Delta z/z \approx 0.25$ for small z . In deriving this relation, two clusters have been excluded because their redshift appeared to be inconsistent (beyond three standard deviations) with the magnitude given in the catalogue. Hereafter co-moving distances $d = D_L(z)/(1+z)$ will always be scaled by the value of $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and quoted in km s^{-1} .

A preliminary identification of candidate superclusters was made using a percolation algorithm on the reference catalogue, that is, the catalogue with distances $d = \langle d \rangle$, where the average is derived from a gaussian distribution in $\log [D_L(z)] + 0.2K(z)$ with variance σ_{sample}^2 . The statistical robustness of these structures was verified on 100 random catalogues in which the estimated redshift for each cluster was drawn according to the previous distribution.

A detailed description of the z estimates and of the supercluster candidates from the ACO catalogue, as well as of their robustness and membership, will be given elsewhere (R.S. *et al.*,

TABLE 1 Clusters within the region delimited by $12^{\text{h}}40^{\text{m}} \leq \alpha \leq 14^{\text{h}}08^{\text{m}}$, $-41^{\circ} \leq \delta \leq -25^{\circ}$, with a mean co-moving distance $\langle d \rangle \leq 20,000 \text{ km s}^{-1}$

Identification no.	α	δ	$\langle d \rangle$	N_{gal}
1736	13 h 24.3 min	$-26^{\circ}54'$	10,225	104
3526	12 h 46.1 min	$-41^{\circ}02'$	3,271	33
3528	12 h 51.6 min	$-28^{\circ}45'$	15,922	70
3530	12 h 52.9 min	$-30^{\circ}05'$	13,494	34
3532	12 h 54.6 min	$-30^{\circ}06'$	14,892	36
3535	12 h 55.1 min	$-28^{\circ}13'$	16,902	30
3537	12 h 58.3 min	$-32^{\circ}10'$	4,945	35
3542	13 h 05.9 min	$-34^{\circ}18'$	17,108	45
3553*	13 h 16.4 min	$-36^{\circ}55'$	15,141	36
3554	13 h 16.7 min	$-33^{\circ}13'$	17,220	59
3555	13 h 18.0 min	$-28^{\circ}43'$	17,645	61
3556	13 h 21.3 min	$-31^{\circ}24'$	16,916	49
3557	13 h 22.1 min	$-28^{\circ}37'$	16,628	36
3558*	13 h 25.1 min	$-31^{\circ}14'$	13,948	226
3559*	13 h 27.1 min	$-29^{\circ}16'$	13,641	141
3560*	13 h 19.0 min	$-32^{\circ}58'$	12,349	184
3562*	13 h 30.7 min	$-31^{\circ}25'$	14,422	129
3564*	13 h 31.5 min	$-34^{\circ}58'$	12,406	53
3565	13 h 33.8 min	$-33^{\circ}43'$	3,241	64
3566*	13 h 36.1 min	$-35^{\circ}18'$	15,361	100
3570	13 h 43.9 min	$-37^{\circ}40'$	10,851	31
3571*	13 h 44.6 min	$-32^{\circ}37'$	15,259	126
3572*	13 h 45.3 min	$-33^{\circ}08'$	12,992	49
3574	13 h 46.3 min	$-30^{\circ}03'$	4,183	31
3575*	13 h 49.7 min	$-32^{\circ}38'$	13,597	49
3577*	13 h 51.5 min	$-27^{\circ}36'$	15,152	103
3578	13 h 54.7 min	$-24^{\circ}29'$	9,969	52
3581	14 h 04.6 min	$-26^{\circ}47'$	11,931	42

Clusters with identification no. in boldface have measured redshifts listed in the ACO catalogue. After this work was completed, we found measured redshifts²⁶ for four of the clusters with distance estimates. The measured values and the estimated ones are in agreement within the errors.

* Clusters tentatively belong to the main complex cited in the text (value of the percolation radius $R = 1,500 \text{ km s}^{-1}$).

manuscript in preparation). Of course, because of the uncertainty in our distance estimates, some numerical details in the following discussion may change after observed redshifts become available.

A remarkable concentration¹⁶ of clusters in the direction of Centaurus is clearly evident in Fig. 1, which shows an all-sky projection of all the ACO and Abell clusters with a measured or estimated distance $< 20,000 \text{ km s}^{-1}$. We have derived an estimate of the overdensity of clusters in this region, which we call the α -region, in the following way. The ACO catalogue covers 1.38 steradian in the latitude range $20^\circ \leq |b| \leq 40^\circ$. In this area it contains 96 clusters up to a distance of $25,000 \text{ km s}^{-1}$. Subtracting the number of clusters and the corresponding volume of the α -region (28 clusters in 0.11 steradian up to a distance of $20,000 \text{ km s}^{-1}$), we retain 68 clusters in a volume of $\sim 7 \times 10^6 h^{-3} \text{ Mpc}^3$, corresponding to a density of $\sim 1 \times 10^{-5} h^{-3} \text{ clusters Mpc}^{-3}$. This would imply an expected number of 2.9 clusters in the α -region, corresponding to an observed overdensity of about a factor of 10 with respect to the mean volume density of all the other ACO clusters at a comparable galactic latitude (see Fig. 2). A possible source of error in the determination of the mean volume density of clusters (in addition to the statistical error which amounts to $\sim 12\%$) could, in principle, derive from an incompleteness of the catalogue near the edge of the volume that we have used. We are, however, confident that this introduces only a minor uncertainty in our estimate, for two reasons. First, it is claimed¹³ that the ACO catalogue is "nominally complete to $z = 0.2$ for clusters with populations of 30 or more galaxies in the magnitude range m_3 to m_{3+2} "; second, an analysis of the density of the ACO clusters as a function of distance (R.S. *et al.*, manuscript in preparation) shows that, after excluding the clusters in the α -region, there is no evidence of a significant decrease of the density of clusters with richness $R \geq 0$ up to at least $R = 30,000 \text{ km s}^{-1}$. In fact, similar values for the mean density of clusters are derived by using the volumes within $R = 30,000 \text{ km s}^{-1}$ (130 clusters) or $R = 35,000 \text{ km s}^{-1}$ (190 clusters).

As seen in Fig. 2, the distribution with depth of the clusters in the α -region is not uniform, but is peaked at $\langle d \rangle \approx 4,000 \text{ km s}^{-1}$ and at $\langle d \rangle \approx 14,000 \text{ km s}^{-1}$. The complex formed by the clusters denoted by '*' among those listed in Table 1, appears to be worthy of future, detailed study. This candidate supercluster consists of a number of members similar to that of the Corona Borealis supercluster¹⁷, but has the advantage of being almost two times closer and two times more dense in clusters with richness $R \geq 1$ than the Corona Borealis supercluster.

We are wary of using the spherical infall model to estimate the possible dynamical influence of the listed clusters, and have computed the peculiar acceleration, A_p , induced on the Local Group (LG) by the clusters listed in Table 1 according to $A_p = A - \langle A \rangle$. Here $\langle A \rangle$ is the acceleration expected from the mean density of clusters in the same volume. It amounts to $< 10\%$ of A , the acceleration induced on the LG by the observed clusters, which has been computed as¹⁸ $A = G \sum_i M_i \hat{r}_i / r_i^2$ where the mass of each cluster ($M_i \equiv M_{R=2}(N_i^{\text{gal}}/106)$) is weighted by the number of galaxies in the cluster, N_i^{gal} , and is expressed in terms of the typical values of an $R=2$ Abell cluster (that is, Coma, for which $N_i^{\text{gal}} = 106$). We also have to assume pure Hubble flow for the proper distance, $r_i = d_i H_0 / (1+z)$, and obviously this can introduce systematic errors because of the presence of coherent peculiar flows.

About half the total computed acceleration is due to the nearest chain of four clusters ($\langle d \rangle \approx 4,000 \text{ km s}^{-1}$, consistent with the quoted distance of the Great Attractor) and the rest is due to the combined pull of the more distant complexes (see Fig. 2), which are dominated by the one at $\langle d \rangle \approx 14,000 \text{ km s}^{-1}$ (eleven clusters denoted by '*' in Table 1). This could help explain part of the 'large' peculiar velocity of the Centaurus cluster⁶ and is consistent with the present lack of detection² of a reversed sign for the peculiar flow beyond $4,500 \text{ km s}^{-1}$. From 1,000 simulations, in which the estimated redshifts of the clusters were randomized around their nominal value, the standard error on the positively skewed distribution of the modulus of A is $\leq 5\%$ whereas, given our narrow cone region, the direction

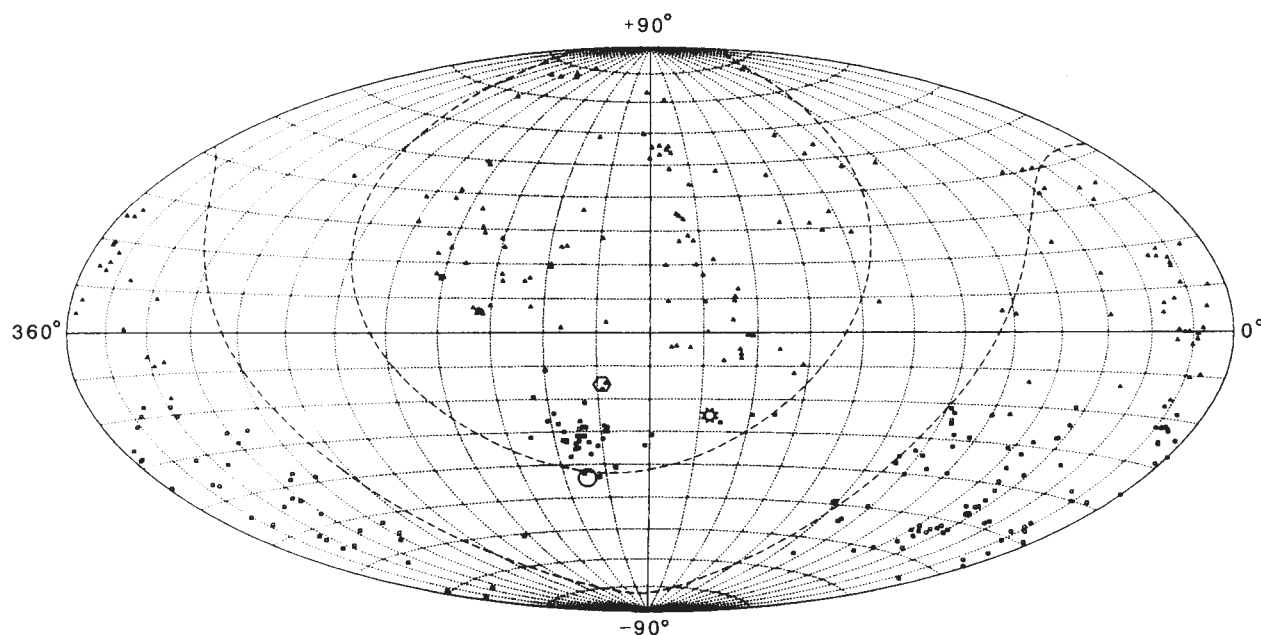


FIG. 1 Equi-area projection in right ascension (α) and declination (δ) of all the rich clusters with measured or estimated distances within $20,000 \text{ km s}^{-1}$. Triangles and squares represent Abell and ACO clusters respectively (some clusters are listed in both catalogues). The star shows the direction of the cosmic microwave dipole⁴, the empty circle shows the

latest direction of the Great Attractor^{5,6}, and the empty hexagon shows the direction of the peculiar flow⁷. Between the latter two symbols the cluster concentration discussed in the text is clearly visible. Dashed lines indicate the avoidance zone due to the galactic plane ($|b| \leq 20^\circ$).

almost coincides with the clusters' barycentre, being always within one degree from right ascension $\alpha = 13^{\text{h}}26^{\text{m}}$ and $\delta \approx -33^\circ$. This agrees very well with one ($\alpha = 13^{\text{h}}20^{\text{m}}$, $\delta = -33^\circ$) of the possible directions for the Supergalactic Centre². It is also close to a previously reported quadrupolar distortion of the peculiar flow¹⁹, while most of the optical dipole signal is due to a patch of sky also centred on the same zone²⁰. Indeed, although the close agreement of the latter dipole with that of the cosmic microwave background favours a 'local' (within $\sim 4,000 \text{ km s}^{-1}$) convergence of the peculiar acceleration, its contribution to the infall towards Centaurus²⁰, $v_{\text{p}}^{\text{dip}} \sim 250 \text{ km s}^{-1}$, seems to account for only half of the observed one, again in good agreement with our above estimate.

If we now make the extreme hypothesis that the component of our peculiar velocity, $v_{\text{p}} \approx 570 \text{ km s}^{-1}$, towards this direction is entirely due to the A_{p} derived above, by using the relation¹⁸ $v_{\text{p}} = \frac{2}{3} H_0^{-1} \Omega_0^{-0.4} A_{\text{p}}$, we have

$$M_{(R=2)} \approx 5 M_{15} h^{-1} \left(\frac{\Omega_0}{0.2} \right)^{0.4} \left(\frac{v_{\text{p}}}{570 \text{ km s}^{-1}} \right) \quad (1)$$

where $M_{15} \equiv 10^{15} M_{\odot}$.

This shows that in an open universe it would be possible to ascribe the whole required peculiar velocity to the observed clusters if the mass overdensity of an $R=2$ cluster were $\sim 5 M_{15}$. Recent estimates²¹ give the mass of the Coma cluster as $\approx 3 M_{15}$. The mass required is undoubtedly high, but the nominal value from equation (1) can be significantly lowered if the overdensity of clusters reported here extends into the avoidance region (see Fig. 1), and if, because of the incompleteness at such low galactic latitudes, many more clusters are present in the region we consider; according to a probability law $P(|b|) = 10^{[-0.3(\cos|b|-1)]}$, one is likely to have missed half of the clusters with $20^\circ \leq |b| \leq 40^\circ$. Moreover, the required mass is not simply the mass confined within the Abell radius, but may be associated with larger galaxy aggregates in which the clusters are embedded, as suggested by the galaxy-cluster cross-correlation^{22,23}. Similarly, if we divide the mass estimates¹⁷ for the Corona Borealis Supercluster, $M_{\text{SC}} \approx 26 M_{15}$, by the number of members ($N_{\text{cl}} = 6$ and weighted as above by the number of galaxies²⁴) we obtain $M_{(R=2)} \approx 6.6 M_{15}$, which is in good agreement with the value derived from equation (1). Of course, this is not the end of the game because a biased distribution of matter would not be consistent with our assumption of clusters as tracers of the mass distribution, although recent estimates²⁵ seem to indicate a global biasing factor not very far from unity. A value of $\Omega_0 = 1$ could still be made consistent with the constraints found from equation (1) either by boosting the estimate for $M_{(R=2)}$ or, more realistically, by not requiring that the peculiar velocity be entirely due to a 'forward' gravitational pull. Underdensities and other great, comparable aggregates (Perseus-Pisces, or another new candidate supercluster similar to the one we present here but at larger distance (R.S. *et al.*, manuscript in preparation)) may have significant dynamical influences.

No definite conclusions can be drawn without a more complete picture of our surroundings. It would be of great interest to determine whether clusters of galaxies are partly responsible for the overall dynamics or, more unlikely, if we are led to infer the existence of very large mass condensations² ($M_{\text{GA}} \approx 54 M_{15}$) in which no very rich clusters are seen³. This, if not attributed to highly specific initial conditions, might imply the existence of a physical process which would have coherently prevented cluster—but not galaxy—formation on scales of $50\text{--}100 h^{-1} \text{ Mpc}$.

Detailed study of this region is very important, not only because no similar nearby concentration of clusters exists elsewhere, but also because of its direction. It is hard to interpret as a mere coincidence the fact that such a concentration of clusters of galaxies is so close in the sky to both the direction of the cosmic microwave background dipole and that derived

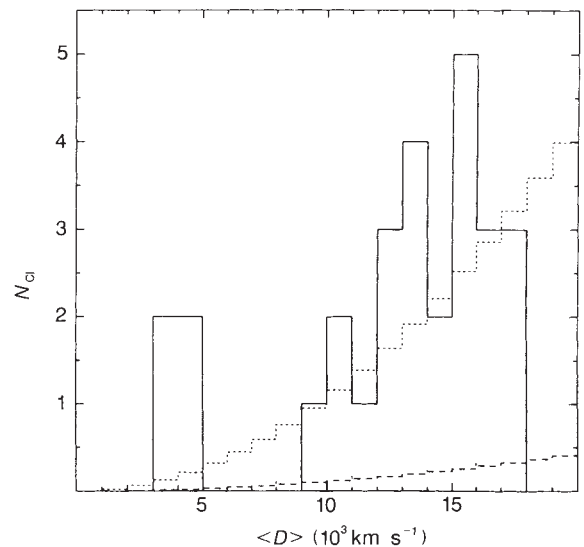


FIG. 2 Distribution in distance of the 28 clusters from Table 1 (solid line). The short-dashed line shows their expected distribution if they were uniformly distributed. The long-dashed line shows the distribution expected from the average cluster density.

from the peculiar motions of galaxies with respect to the Hubble flow. But it must be said that the large-scale mass fluctuation necessary if the whole peculiar velocity is due to the clusters in Table 1 would be difficult to accommodate in many current cosmological models.

Further studies of clusters in this area with intrinsic distance estimates should eventually find the distance at which the peculiar flow reverses its sign, thus delineating the region which contains the bottom of a great potential well (assuming that this is the major cause of the reported peculiar velocities (A. A. Starobinsky, preprint)). The concentration discussed here appears to be the best candidate for the bottom of such a well because of both the number of clusters present and their particular richness. $\square$

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Asymmetry of the envelope of supernova 1987A

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THE supernova SN1987A in the Large Magellanic Cloud has been observed by high-angular-resolution speckle interferometry since 25 March (30 days after the explosion) with the 4-m telescope at the Cerro Tololo Interamerican Observatory. These observations have provided a number of results that may be central to a detailed understanding of this unique event. Data obtained on 25 March and 2 April 1987 revealed a second bright 'companion' source separated from the supernova by 60 milliarcseconds and less than three magnitudes fainter than the supernova¹. Measurements of the average diameter of the supernova envelope have been made from data recorded from March 1987 to April 1988². Here we present a more detailed analysis of these data, which shows that the expanding envelope is asymmetric. This asymmetry has been observable since June 1987. The ratio between the minor and major axes of the envelope profile is about 2–3, and the position angle of the major axis is $20^\circ \pm 5^\circ$, consistent with results reported from polarization measurements. The major axis is aligned with the position angle of the companion to the supernova.

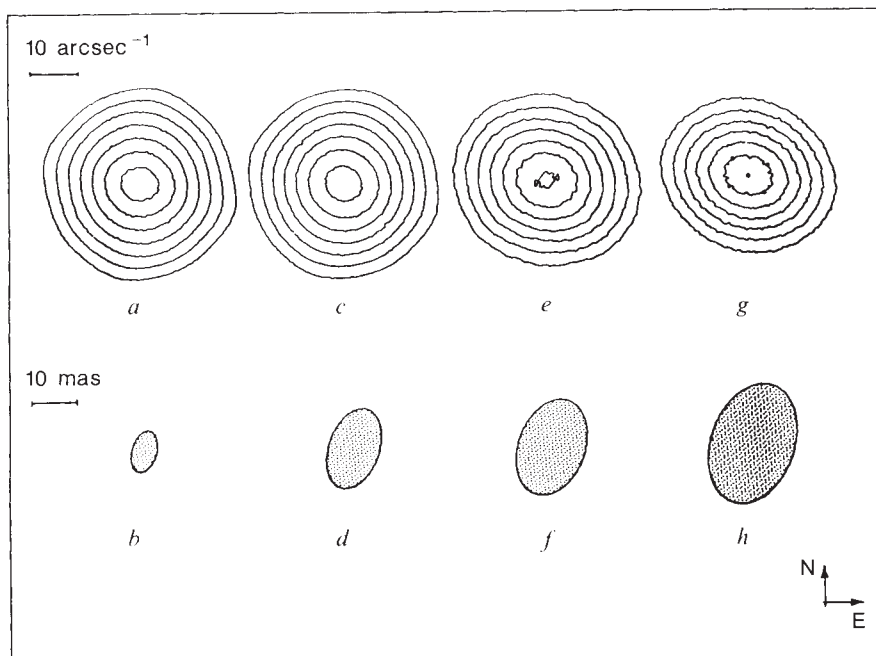
Speckle observations at optical wavelengths have been carried out using the PAPA detector³, a two-dimensional photon-counting sensor which records a catalogue of successive photon positions. The detector currently has a maximum data recording rate of 100,000 photon s^{-1} in a field of 512×512 pixels. The speckle camera uses a fore-optics package which provides magnification of the image, so that the diffraction-limited scale of the telescope is matched to the pixel size of the camera, narrow-band optical filtering, which yields temporal coherence sufficient to allow interference over the path-length errors introduced by atmospheric aberrations, and atmospheric-dispersion correction, using a computer-controlled prism compensator. The digital output of the camera, in the form of photon addresses, is encoded on a video carrier and recorded with a VCR. The

stored addresses are recovered in the laboratory and unpacked for the speckle processing.

The photon addresses are grouped into frames, with a short time per frame relative to the atmospheric correlation time. This approach allows framing of the data for maximization of the signal-to-noise ratio in the integrated result; typical frame times range from 2 to 20 ms. Individual frames are built from the photon list by incrementing the number in the array position corresponding to the address of each detected photon. Corrections for the camera sensitivity (flat fielding) are made on the resulting frames. The Fourier transform for each frame is then calculated and accumulated into the power spectra and the complex correlation arrays which are required for diameter measurement and speckle image reconstruction using our version of the Knox-Thompson image-reconstruction algorithms⁴. Compensation for the atmospheric and telescope transfer function is accomplished by observing an unresolved comparison star immediately before and after the object observations. This star is chosen to be as close in angular position to the object as possible so that its atmospheric statistics are similar. Deconvolution by the reference star enhances the amplitudes of the high angular frequencies in the reconstruction. A detailed treatment of the algorithms and data processing is discussed elsewhere⁴.

Data sets on SN1987A and other stars result in diameter determinations with a precision of better than one milliarcsec. These measurements are made by fitting the integrated power spectra to the power spectrum of a uniform disk², and, depending on the signal-to-noise ratio, can give results with resolution exceeding the diffraction limit of the telescope. Further analysis of the speckle data recorded between June 1987 (95 days after the explosion) and April 1988 (411 days after the explosion) shows that the expanding shell is elongated⁵. This asymmetry is detected from data recorded at several wavelengths between 442.0 nm and 850.0 nm. Figures 1a, c, e and g show the power spectra of SN1987A obtained from the data recorded near the centre of the H α emission line in May–June 1987, November 1987, February–March 1988 and April 1988. These power spectra are elongated, with a major axis corresponding to a position angle (PA) in image space of 20° (or 200°) $\pm 5^\circ$. Similar departures from circular symmetry are seen in the power spectra obtained from data recorded in several different wavelengths. By fitting the power spectra of uniform-brightness ellipses to the observed power spectra, we determined the lengths of the

FIG. 1 Speckle power spectra of SN1987A recorded in H α at four different epochs, (a, c, e, g) and ellipses showing schematically the size and orientation of the envelope at each epoch (b, d, f, h). a, b, June 1987; c, d, November 1987; e, f, February 1988; g, h, April 1988.



major and minor axes, and their position angles. All of the measurements gave the same PA within the error bars. A summary of the measurements is given in Table 1. In contrast, data recorded on comparison stars give symmetric power spectra and symmetric images with measured angular diameters of less than 3 mas.

The ratio of the minor to major axis of the supernova's envelope is about 2-3 for all four data sets. The apparently increasing asymmetry in the supernova's power spectra shown in Fig. 1 is due to the increasing angular size of the supernova relative to the telescope's diffraction limit. Figures 1*b*, *d*, *f* and *h* show the ellipses whose Fourier transforms are fitted to the power spectra at the various epochs.

True images of SN1987A and its comparison star, ν Vol, were reconstructed using Knox-Thompson speckle-imaging techniques⁴. Figure 2 shows the images from data recorded in April 1988 at 533.0 nm (10-nm bandpass) and H α (10-nm bandpass). The elongation of the supernova images, along an axis inclined at a position angle of 20° (or 200°), is quite evident despite the fact that its angular size in April was only slightly greater than the telescope's diffraction limit. Because the size of the image was so close to the telescope diffraction limit, the size of the axes were determined from power spectra, not from reconstructed images. The images also appear to be somewhat brighter in the south-west direction. The ν Vol images show no elongation and are symmetric. Their size is equivalent to the beam size for the reconstruction process.

In addition to our speckle results, other observations of SN1987A show strong evidence for an asymmetrically expanding shell⁶. Early spectroscopic observations (between 20 and 80 days after the explosion) detected a double-peaked feature in several hydrogen lines at optical and infrared wavelengths⁷⁻⁸. The appearance of two equally displaced components at longer and shorter wavelengths than the maximum emission was interpreted as being due to some departure from spherical symmetry of the shell.¹⁰ Polarimetric observations¹¹⁻¹⁴ also show strong evidence for asymmetry in the envelope with a position angle of $\sim 200^\circ$. The observed polarization structure was interpreted as arising from an asymmetrically expanding, scattering atmosphere. Polarimetric data were modelled by a scattering atmos-

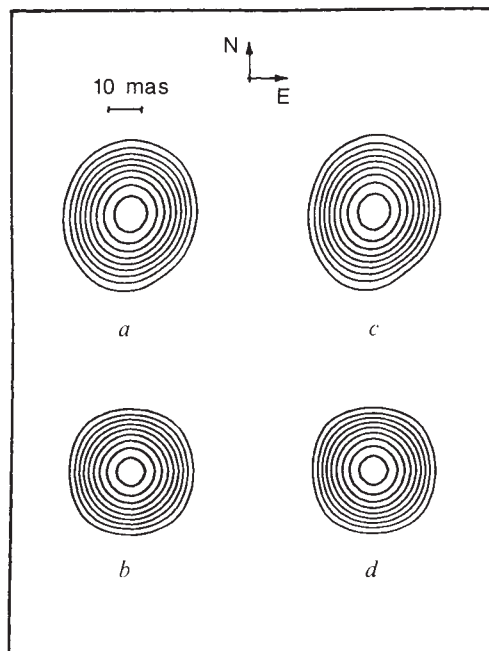


FIG. 2 Image reconstructions of SN1987A and a comparison star recorded in April 1988. *a*, SN1987A at 533.0 nm. *b*, Comparison star at 533.0 nm. *c*, SN1987A at H α . *d*, Comparison star at H α .

TABLE 1 Measured asymmetries for SN1987A (PA = $200 \pm 5^\circ$)

Days after explosion	λ (nm)	Average diameter (mas)	Major axis (mas)	Minor axis (mas)
95-98 (May-June 87)	5,330	18 ± 2	19 ± 2	17 ± 2
	6,565 (H α)	8 ± 1	10 ± 2	6 ± 2
	7,750	15 ± 2	20 ± 2	10 ± 2
265-268 (Nov. 87)	6,585 (H α)	17 ± 1	18 ± 3	14 ± 3
	8,500	16 ± 1	20 ± 2	14 ± 2
370-373 (Feb.-Mar. 88)	4,420	25 ± 2	27 ± 2	22 ± 2
	5,330	20 ± 1	24 ± 2	14 ± 2
	6,400	17 ± 1	20 ± 2	15 ± 2
	6,565 (H α)	21 ± 1	24 ± 2	16 ± 2
	7,000	17 ± 1	20 ± 2	16 ± 2
	8,500	18 ± 2	25 ± 2	12 ± 2
409-411 (April 88)	5,330	26 ± 2	30 ± 2	20 ± 2
	6,565 (H α)	27 ± 1	30 ± 2	20 ± 2
	8,500	24 ± 2	28 ± 3	16 ± 3

phere shaped as a prolate or oblate spheroid^{12,13,15}. Best fits have been obtained for ratios of the smallest to the largest axis of these spheroids ranging from 0.6 to 0.9. Recently, γ -ray observations have yielded linewidths requiring either fragmentation or asymmetry of the supernova shell for their explanation¹⁶.

Present results from speckle interferometry, polarimetry, spectroscopy and γ -ray observations show that the expanding atmosphere of SN1987A is not spherically symmetric. The theoretical models that assume spherical symmetry must clearly be revised. An asymmetric envelope may also be necessary to explain, for example, the early emergence of X-rays and γ -rays and the evolution of their spectra with time¹⁷. Woosley¹⁸ has suggested that non-uniformities in the collapse and core bounce could produce an asymmetric explosion, but that this asymmetry is far less likely to propagate to the outer envelope. Chevalier and Soker¹⁹ have modelled the expanding supernova envelope and concluded that the most likely mechanism for asymmetry is rotational flattening of the progenitor envelope, probably requiring a binary companion during stellar evolution. Much of the evidence for asymmetries and extended structures around the supernova is summarized by Trimble²⁰. □

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Deuterium content of the Venus atmosphere

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THERE is no liquid water on Venus. The water vapour in its atmosphere would, if condensed, form a layer only 20 cm deep which means, in contrast to the 3-km-deep oceans that cover its sister planet Earth, that Venus is very dry indeed. It is not known with certainty whether Venus has lacked water since its formation, or if water once present has been lost during its lifetime; the question is of special interest as water is generally thought to be a necessary ingredient for the development of life. The abundance of deuterium in the atmosphere of Venus is an important clue to the planet's history, because ordinary and deuterated water escape at different rates. Using the high-resolution mode of the International Ultraviolet Explorer (IUE), we measured hydrogen Lyman- α -emission but found only an upper limit on deuterium Lyman- α -emission, from which we inferred a D/H ratio of less than $2\text{--}5 \times 10^{-3}$. This is smaller by a factor of 3–8 than the D/H ratio derived from measurements by the Pioneer Venus Large Probe, and may indicate either a stratification of D/H ratio with altitude or a smaller overall ratio than previously thought.

The Pioneer mass spectrometer detected a signal at mass 19 (HDO^+), when the inlet became clogged by a droplet of sulphuric acid. The detection was taken¹ to imply a D/H ratio of 1.6×10^{-2} . If this modern ratio derives from an original D/H ratio similar to Earth's, that is, 1.6×10^{-4} , in sea water, but enriched by differential evaporation, Venus would have had enough water to form an ocean 20 m deep, although this is an extreme lower limit, because some deuterium would undoubtedly have escaped too.

The presence of HDO in the atmosphere of Venus will produce some D atoms by photodissociation and some consequent D Lyman- α -emission in the planet's ultraviolet airglow. In the upper atmosphere, the Lyman α transition of H and D atoms can be excited by resonance scattering of solar photons, which can be observed by remote sensing from outside. The emission lines are separated by $0.33 \text{ \AA}$ (H emission is at $1,215.66 \text{ \AA}$, and D at $1,215.33 \text{ \AA}$), which is much more than the thermal width ($\sim 10^{-2} \text{ \AA}$) but less than the solar linewidth, so that both lines can be excited to an approximately equal extent.

The D Lyman- α -emission has been measured previously² in the upper atmosphere of the Earth with a dedicated Ly α spectrometer on board Spacelab 1, and was found to have an intensity of 300 rayleigh along a tangential line of sight at an altitude of 110 km, just above the O_2 absorption level. The corresponding D/H ratio was found to be $\sim 3 \times 10^{-4}$, indicating a twofold enrichment in D relative to sea water which results from the smaller exospheric escape for D atoms than for H atoms.

The International Ultraviolet Explorer (IUE), launched in 1978, has in principle the spectral resolution required to separate both D and H Ly α lines, and it was therefore proposed that it be used to detect deuterium in the upper atmosphere of Venus at the $\sim 1\%$ level for D/H ratio following the Pioneer mass spectrometer measurement of HDO in the lower atmosphere.

A series of high-dispersion SWP (short-wavelength primary camera) spectra of the Venus dayside disk has been obtained with IUE, both in the course of this programme and in others. Measurements of the IUE echelle-grating scattered-light profile have shown that scattered H Ly emission $0.33 \text{ \AA}$ from the line centre—that is, where D Ly α emission should occur—is well below the level of 10^{-2} expected for such emission³, so that the

detection of D Ly α becomes a problem of signal-to-noise ratio. In principle, the small aperture of IUE (circular, $3.2''$) would give the best separation of D Ly α and H Ly α spectral lines. However, the large aperture ($23 \times 10''$) has a throughput on an extended source that is greater than that of the small aperture by a factor 30, and it was found that large-aperture SWP spectra of the longest duration (that is, with the H Ly α emission feature saturated) provided the best measurement of D Ly α , despite the slight overlap of the D and H features, because of the extended size of the aperture. D emission should be apparent as an asymmetric lineshape (specifically, as a short-wavelength shoulder; see Fig. 1).

Some of the archived spectra were taken with only part of the aperture imaging the disk of Venus, leading to a distorted Ly α lineshape. The photowrite images of all spectra were therefore examined so as to select only those images for which the aperture was filled. A customized reduction method was required to analyse these spectra, because the aperture width used to sum the emission region in the standard IUE reduction is designed to separate the orders of the echelle and is too narrow to include all of the emission entering the large aperture. The Venus spectra contain discrete Ly α emission with no adjacent continuum other than longer-wavelength light scattered by the cross-disperser grating across the region of Ly α . In the customized reduction we therefore (1) summed the entire emission region of the Ly α lines; (2) employed a more extended region of background measurement for an accurate removal of the scattered light; (3) re-processed all images with VAX floating-point accuracy in the intensity transfer functions using the SDPS processing routines at Goddard Space Flight Center; and (4) carefully aligned all spectra in wavelength to the observed centre of the H Ly α line before summing. Thermal drifts of the spectrum across the detector face with time are advantageous, as the fixed noise pattern of the SEC cameras is altered when the images are realigned in wavelength and the noise level is therefore reduced in the summed spectrum.

The longest archived exposures of Venus lasted for 60 min: exposures longer than this were precluded by extreme saturation of the camera at the long-wavelength end of the spectrum. Six spectra ranging in duration from 30 to 60 min were summed as described and the result is plotted in Fig. 1. These spectra were obtained near the Venus maximum elongation, on 12 and 13 April and 26 August 1980. The Ly α intensity, estimated from other shorter, unsaturated exposures, was found to be

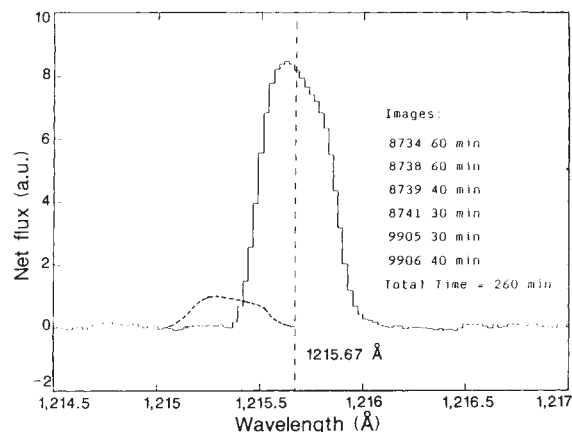


FIG. 1 Hydrogen Ly α spectra of bright-disk emission from Venus, obtained with the IUE large aperture and summing the six best images (solid line). The line intensity corresponds to 21×10^3 rayleigh; the long-dashed line shows the line centre. The short-dashed line shows the profile calculated for deuterium Ly α emission of 2.5×10^3 rayleigh (corresponding to a D/H ratio of 1.6×10^{-2}). On the basis of the IUE observations, we can rule out such emission down to a factor of ~ 8 times less than this. The numbers refer to spectra reference numbers and exposure times. a.u. denotes 10^5 IUE flux units.

$(21 \pm 5) \times 10^3$ rayleigh, which is consistent with the Pioneer Venus Orbiter ultraviolet spectrometer (PVO UVS) observations^{4,5}. Although there is some saturation for the images summed in Fig. 1, the IUE procedure is able to account for this moderate saturation because intensity for the average spectrum is the same as that found for unsaturated exposures. The dashed line in Fig. 1 shows D Ly α emission of 2.5×10^3 rayleigh modelled by scaling down the observed spectrum by a factor of 8 and displacing it by 0.33 Å. As such a shoulder does not appear in the observed spectrum, there seems to be no significant D Ly α emission; an examination of the noise level in this region allows us to place an upper limit (2σ) of 300 rayleigh for such an emission. Although this implies an upper limit of 1.5% on the ratio of intensities, we shall see below that it implies a substantially lower D/H density ratio.

In the upper atmosphere of Venus, D and H atoms are excited by the solar Lyman α line at about the same rate. However, CO₂ absorbs Lyman α at an altitude of 110 km, and only those atoms above this level contribute to the emerging intensity. The IUE upper limit (2σ) of $I_D = 300$ rayleigh implies an upper limit for the vertical column density, N_D , of D atoms above 110 km of $N_D = I_D \times 10^6 / g_d$, where g_d is the excitation rate of D atoms. The ratio g_d / g_h (with g_h defined in similar fashion for H atoms) can be estimated from the relative flux intensities at the solar line centre, F_s (H excitation), and at D resonance (displaced from line centre by 0.33 Å); these measurements have been made by OSO-5 (ref. 6) (Orbiting Solar Observatory), and indicate a ratio of 0.8. The value of g_h may be found from $g_h = 0.544 \times 10^{-14} F_s s^{-1}$, with $F_s = 8 \times 10^{11}$ photon $cm^{-2} s^{-1} \text{Å}^{-1}$ at Venus (ref. 4). Variations in F_s over the 2-year period of IUE observations were smaller than $\pm 10\%$ (ref. 5), this being confirmed by monitoring of the total solar Ly α flux from Earth's orbit by SME. Thus, $g_h = 4.35 \times 10^{-3} s^{-1}$, so that $g_d = 3.5 \times 10^{-3} s^{-1}$ and, for $I_D = 300$ rayleigh, we obtain $N_D = 8.6 \times 10^{10}$ atom cm^{-2} . The photometric uncertainty of IUE at the Ly α wavelength is estimated as $<25\%$; indeed, the H Ly α intensity of 21×10^3 rayleigh measured by IUE agrees well with the nadir, subsolar value of 20×10^3 rayleigh measured by PVO UVS at the same time^{4,5}.

The ratio I_D / I_H from the disk of Venus is different from the D/H density ratio in the lower atmosphere, mainly because the atmosphere above 110 km is optically thin at Ly α for D atoms but is moderately thick for H atoms, so that here I_D / I_H is larger than the ratio of column densities N_D / N_H . Because of the nonlinear dependence of I_H on N_H , one cannot derive N_H directly from these IUE observations. Thus to estimate N_H we use instead the Pioneer observations analysed by Paxton *et al.*^{4,5}; these were dayside observations, made not far from the subsolar point, and therefore are pertinent to the present IUE observations.

The PVO UVS was better situated than IUE to determine an absolute value of the H density in the Venus atmosphere, because the Pioneer measurements were performed both from outside and from inside the H atmosphere, and also because the spinning geometry of PVO UVS permits measurement of bright disk intensities as well as limb profiles. The H medium is optically moderately thick so that the shape of the Ly α variation over one spacecraft spin depends upon the absolute H concentration everywhere between 110 km and the outside exosphere. The H density vertical distribution can therefore be determined without knowledge of either the Ly α centre solar flux or the UVS calibration⁴, by comparing radiative transfer predictions, made on the basis of a wide range of models of H density distribution, with the observations.

Paxton *et al.*⁵ use two parameters to describe the H vertical profile: the exobase density n_e at 200 km, and the escape flux ϕ_e (also at 200 km), which, for a given n_e , influences the density at the homopause level. The PVO UVS results indicate that, over the two-year period of observations (May 1979 to May 1981), the H profile near the subsolar point is remarkably con-

stant and can be described by $n_e = (6 \pm 1.5) \times 10^4 \text{ cm}^{-3}$ and $\phi_e = 7.5 \pm 1.5 \times 10^7 \text{ cm}^{-2} s^{-1}$. The corresponding column density N_H above 110 km was determined to be $(2.4 \pm 0.8) \times 10^{13} \text{ cm}^{-2}$ (ref. 5).

Combining the various uncertainties quadratically gives an upper limit (2σ) to the ratio N_D / N_H of $(3.6 \pm 1.5) \times 10^{-3}$. The ratio of densities n_D / n_H at some reference level (say, 100 km) will be slightly different from the value of N_D / N_H , because the D and H vertical profiles are not identical. Integrating the equation of diffusion⁷ for D atoms using the VIRA atmospheric profile⁸ we obtain the relation $n_D / n_H (100 \text{ km}) = \alpha (N_D / N_H)$. If the escape flux of D atoms is zero then $\alpha = 0.55$; if it is equal to the H escape flux (relative to the exobase density at 200 km) then $\alpha \approx 1$, and if it is 0.32 to make a rough allowance for the mass fractionation effect, then $\alpha \approx 0.97$.

Therefore, regardless of the value assumed for the D escape rate, N_D / N_H above 110 km is not very much different from the D/H ratio below the homopause. This is to be expected, as the homopause occurs at around 130 km well above the penetration level of Ly α in CO₂. The upper limit of D/H at 100 km of $\sim (3.6 \pm 1.5) \times 10^{-3}$ is lower by a factor of 4.4 than the value previously reported for the lower atmosphere¹, and in fact is probably closer to 2×10^{-3} if present-day deuterium escape is negligible, which is entirely possible.

This low value can be compared with another set of measurements made by the Pioneer Venus Orbiter ion-mass spectrometer. Ions of mass 2 were originally identified as H₂⁺ ions,⁹ but were later assigned to D⁺ (refs 10, 11). A detailed ionospheric model¹² of the pre-dawn bulge ionosphere supported this assignment to D⁺ but showed that it required D/H neutral-atom ratios at the homopause of 1.4 and 2.5% respectively for the two orbits during which measurements were made. Clearly, the non-detection of D Ly α emission in the IUE data argues against such a quantity of deuterium at the homopause, suggesting that the mass-2 ions may indeed have been H₂⁺ ions. To obtain an H₂⁺ signal of the magnitude observed would require an H₂ abundance of 10 p.p.m. on Venus¹³, which is consistent with previous gas-chromatograph data from Venera 13–14 (ref. 14).

Despite the apparently small content of deuterium in the upper atmosphere of Venus, it is possible that more substantial amounts exist in the lower atmosphere—as suggested by the identification of a mass-19 signal, measured by the Neutral Mass Spectrometer, with HDO⁺—as a result of some atmospheric fractionation process. On the other hand, Spacelab-1 results have shown clearly that such processes do not operate on the Earth, and given that the aeronomy of H₂O, H₂ and H does not seem to differ greatly for the two planets, they would not be expected to occur on Venus either. It seems possible, therefore, that the D/H ratio measured in the upper atmosphere of Venus does indeed represent the bulk atmospheric ratio. The Hubble Space Telescope will provide the means to test these results, as its High Resolution Spectrometer will be capable of resolving a D/H ratio of 1.6×10^{-4} (the value on the Earth) on Venus. □

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Tripolar vortices in a rotating fluid

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THE emergence of coherent flow structures is a well-known feature of quasi-geostrophic or two-dimensional turbulence, and because of their relevance to large-scale geophysical flows the dynamics of these structures have been studied increasingly over the past decade¹⁻⁵. The most common coherent structures are the axisymmetric (monopolar) vortex, with circular streamlines, and the vortex dipole, both of which have been found to arise in a variety of situations under different (sometimes nondescript) forcing conditions. The emergence of vortex dipoles, for example, has been observed in turbulence in soap films³, in electrically forced magnetohydrodynamic flows⁵ and in collapsing turbulence in a continuously stratified fluid⁶. There is growing evidence^{4,7-9} that, in addition to the monopolar and dipolar vortices, a tripolar coherent flow structure exists. Here we describe a laboratory experiment in which a tripolar structure is found to emerge from an unstable cyclonic vortex in a homogeneous rotating fluid. The tripole seems to be a very stable structure, even persisting in a highly sheared fluid environment, and might therefore be found in natural geophysical flows.

Our experiment is part of a larger study of the dynamics of barotropic vortices in a rotating fluid¹⁰. The laboratory set-up (Fig. 1) consists of a cylindrical perspex tank (92.5 cm in diameter and 30 cm deep) mounted concentrically on a turntable, whose angular speed is continuously adjustable from 0 to 10 r.p.m. The tank is filled with a homogeneous fluid, and after the table has been set in steady motion, the fluid is spun-up to solid-body rotation. For these experiments the spin-up timescale is typically 40 times the rotation period of the turntable, and the Ekman number based on the water depth is small, typically $\sim 10^{-5}$. Both cyclonic and anticyclonic vortices were created by briefly stirring the fluid confined in a bottomless cylinder placed at the centre of the tank. After allowing the stirring-induced flow to become a purely azimuthal flow, the inner cylinder is withdrawn vertically, releasing the vortex into the solidly rotating ambient fluid. In most experiments the Rossby number associated with the initial vortex flow—based on the vortex diameter and the maximum velocity—was large, that is, of order unity. The resulting motions were recorded photographically by a remote-controlled co-rotating camera mounted above the free surface. The flow of the initially confined fluid was traced by the addition of dye, and streak photography of small tracer particles floating on the free surface provided quantitative information on the total velocity field. Because of the two-dimensionality of the flow, the motion at the free surface, as measured from the streak lengths, is representative of the flow at lower levels.

The evolution of a cyclonic stirring-induced barotropic vortex is illustrated by the sequence of photographs shown in Fig. 2. Immediately after releasing the vortex by lifting the inner cylinder, turbulent mixing is observed in the highly sheared region at the circumference of the vortex (Fig. 2a). Within a few rotation periods, however, the flow settles in an approximately circular vortex with a smoother edge. A slightly non-axisymmetric internal structure becomes established in the dyed fluid (Fig. 2b, c), and eventually the vortex exhibits a tripolar structure, with cyclonic motion in the core and anticyclonic motion concentrated in the two satellite vortices (Fig. 2d, e, f). The tripole structure rotates in a solid-body-like manner around its centre, in a cyclonic direction relative to the rotating frame, as can be seen in the successive photographs. The motion in the interior of the tripole decays slowly, as does the overall rotation speed.

This decay is caused partly by bottom friction and partly by lateral entrainment of initially quiescent ambient fluid into the satellite vortices (visible in Fig. 2d and f). Initially (that is, just after release of the cyclonic stirring-induced vortex, Fig. 2a) anticyclonic vorticity is confined to a narrow region around the core of the cyclonic vortex. The presence of this vorticity can be inferred from the shearing of the dye filaments that get wrapped around the central structure (Fig. 2b). The anticyclonic vorticity gradually becomes concentrated in the two anticyclonic satellite vortices, an effect associated with the growth of a perturbation of azimuthal wavenumber $m = 2$. In many stability studies (for example, refs 11 and 12) this is found to be the fastest-growing mode for unstable circularly symmetric vorticity distributions.

In some additional experiments barotropic vortices were generated in a different way, by taking the fluid level inside the inner cylinder (Fig. 1) lower than that outside. When the cylinder is lifted, gravitational collapse results in an axisymmetric swirling motion, as would be expected from conservation of angular momentum. Although the flow immediately after the collapse is very turbulent, the motion becomes purely azimuthal typically within two rotation periods. These experiments¹⁰ revealed that the vortices remain axisymmetric for relatively large values of $\bar{H}$ and/or $\Delta H/\bar{H}$ ($\bar{H}$ being the mean water depth and ΔH the difference in level), whereas a gradual transition towards a tripole structure was observed for relatively small values of both $\bar{H}$ and $\Delta H/\bar{H}$. For these collapse-induced cyclonic vortices the timescale associated with the tripole formation is considerably longer than for those created by the stirring technique. This is probably a consequence of the different vorticity distributions of each type of vortex: the ring of anticyclonic vorticity encompassing the cyclonic-vorticity core is much wider for the collapse-induced vortices than for the vortices induced by stirring.

Quantitative measurements of the velocity field associated with the tripolar vortex were obtained by streak photography of tracer particles floating on the free surface; a typical example is shown in Fig. 3. Such measurements allow calculation of the spatial distribution of the vorticity ω and the streamfunction Ψ ; the relationship between these quantities—when measured in a reference frame co-moving with the tripole—provides important information about the tripole dynamics. Although in many theoretical studies¹³⁻¹⁶ of (monopolar and dipolar) coherent flow structures a linear relationship between ω and Ψ is assumed, laboratory experiments⁵ on magnetohydrodynamic vortex dipoles indicate the existence of a nonlinear relationship. Our preliminary laboratory measurements⁸ of the digitized velocity field of tripole structures also suggest nonlinearity in the (ω, Ψ) relationship; further details will be published elsewhere. The information provided by digitized streak photographs should be essential for a theoretical description of tripoles.

Although in a 'real' tripole the vorticity is distributed continuously over a compact region consisting of three aligned

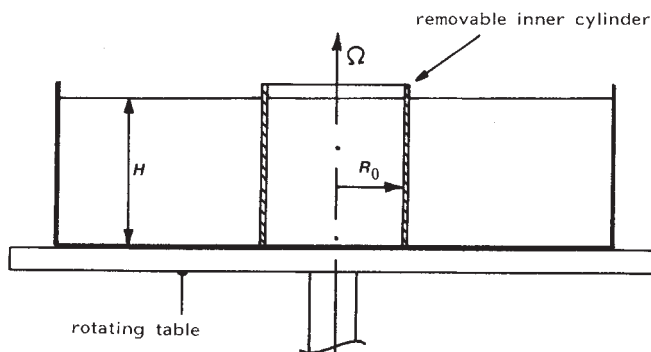


FIG. 1 A schematic drawing of the laboratory set-up used to produce barotropic vortices in a rotating fluid.

patches (the two satellites and the central region, containing anticyclonic and cyclonic vorticity respectively), we can model a tripole crudely in terms of three equidistantly aligned point vortices with circulations of -1 , 2 and -1 , respectively. Such an idealized configuration of vortices exhibits a steady cyclonic rotation around the central vortex, as observed for the laboratory tripoles. The streamfunction of the flow associated with a rotating point-vortex tripole relative to the rotating frame has been calculated⁸, and the corresponding pattern of streamlines (Fig.

4) shows good agreement with the dye patterns observed in the laboratory (Fig. 2*d* to *f*). But because no information about, say, the relation of the circulation of the laboratory tripole to its overall rotation rate is available yet, the quantitative value of such a point-vortex model is not clear at present.

Motivated by their interaction properties, it has been speculated^{17,18} that isolated vortices, that is, monopolar and dipolar structures, are the 'particles' of geophysical fluid dynamics, in the sense that, although interacting, they are able to maintain

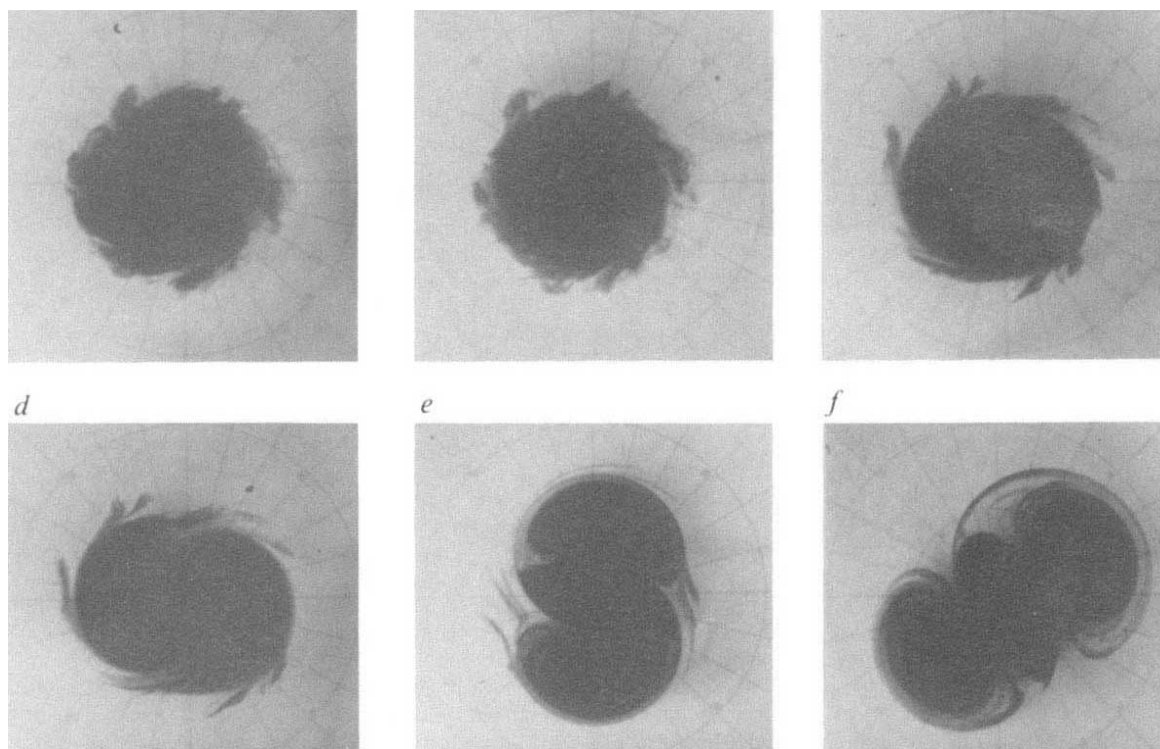


FIG. 2 A sequence of plan-view photographs illustrating the emergence of a tripolar flow structure from a cyclonic barotropic stirring-induced vortex. The photographs were taken at times of *a*, $1.2 T$, *b*, $2.4 T$, *c*, $7.1 T$, *d*, $8.9 T$,

e, $10.7 T$ and *f*, $23.8 T$ after releasing the vortex, with $T=8.4$ s, the rotation period of the turntable. The mean water depth, H , was 14.5 cm, and the initial vortex diameter, $2R_0$, was 11.0 cm.

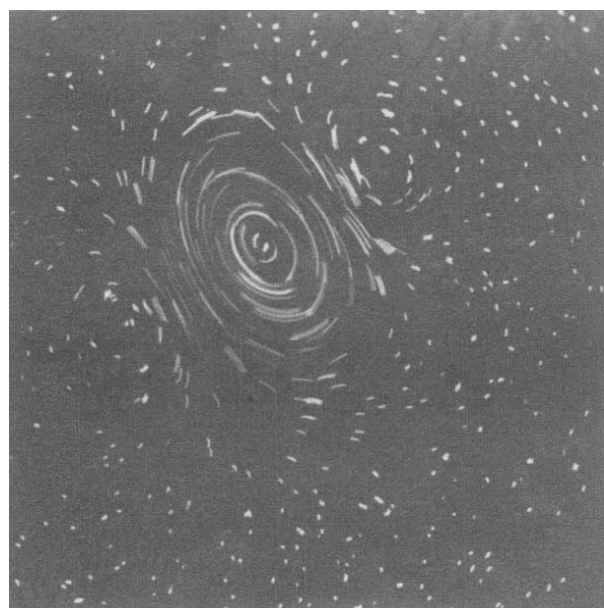


FIG. 3 A typical streak-line photograph of the relative flow field associated with a slowly rotating tripolar vortex.

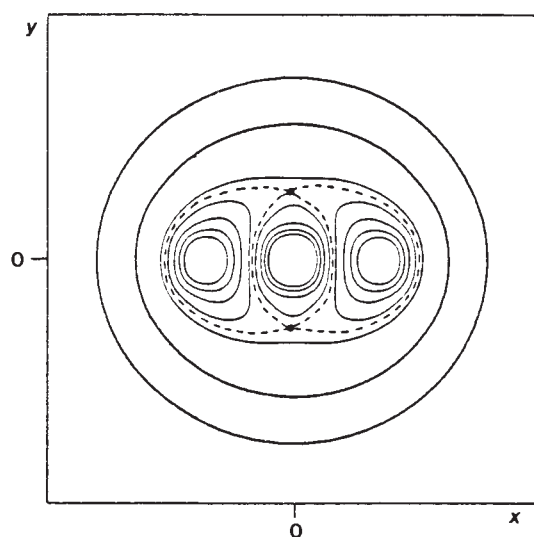


FIG. 4 Theoretical streamline pattern of a rotating tripolar vortex modelled⁸ as three equidistantly aligned point vortices with circulations -1 , 2 and -1 .

their structural identity much better than can dispersing waves. Monopoles and dipoles can be considered as basic flow structures in two-dimensional flows; the former are stationary features containing net angular momentum, whereas dipoles are steadily translating vortex structures that contain net linear momentum. The tripolar vortex, as found in these laboratory experiments and also in numerical simulations of two-dimensional turbulence⁴, appears to be an equally basic flow structure, characterized by a net angular momentum and a steady rotation of the structure as a whole. Its observed persistence in sheared fluid environments suggests that the tripole may be a robust and stable vortex structure. Tripoles have not yet been observed in large-scale geophysical flows, but in view of this apparent stability it is not unlikely that oceanic examples will be found in the near future. □

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Compositional convection in viscous melts

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DURING solidification of multi-component melts, gradients in temperature and composition develop on different scales because of the large difference between their respective molecular diffusivities. Two consequences are the development of double-diffusive convection¹ and the creation of mushy zones in which solid and liquid intimately coexist with a complex small-scale geometry^{2,3}. Theoretical analysis requires simplifying assumptions that must be verified by laboratory experiments. Hitherto, experiments have been carried out with aqueous solutions which do not accurately represent the dynamics of melts with high Prandtl numbers, such as magmas. Here we describe the characteristics of compositional convection using a new experimental technique which allows the viscosity of the solution to be varied independently of chemical composition and liquidus temperature. A super-eutectic melt was cooled from below, causing the growth of a horizontal layer of crystals. Convective instability occurred when the local solutal Rayleigh number of the compositional boundary layer ahead of the advancing crystallization front attained a value of ~ 3 on average. We observed a novel regime of convection in which the thermal boundary layer above the crystallization front was essentially unmodified by the motion of the plumes. The plumes carried a small heat flux and did not mix the fluid to a uniform temperature.

We have carried out experiments with aqueous solutions of ammonium chloride (NH_4Cl) plus small amounts of

hydroxyethylcellulose compound. These low concentrations (<2 wt%) of the latter do not affect the phase diagram but nevertheless allow the solution viscosity to be varied by several orders of magnitude (Fig. 1). The solution was placed in the experimental tank and maintained at a uniform temperature above the liquidus. The base plate of the tank was connected to a pre-cooled bath at time $t=0$, and reached a constant temperature (below the liquidus) after a short transient. Temperatures in the fluid were measured with a vertical array of thermocouples. A few minutes after cooling began, small crystals of NH_4Cl were observed, distributed randomly at many places across the base. Growth continued by the lateral expansion of crystal patches and new nucleation until a mushy layer with a horizontal upper surface became established. Figure 2 shows the thickness of the layer as a function of time.

Our solutions contained concentrations of NH_4Cl greater than that of the eutectic point and hence fractional crystallization produced a boundary layer of light residual liquid. Compositional convection was eventually observed in the form of a field of plumes rising from the crystallizing region (Fig. 3a). The plumes consisted of cylindrical conduits and rounded caps, similar to 'diapir' plumes⁴. Plume cap diameters were in the range 0.3–1.0 cm, that is, substantially larger than the crystal size (<0.1 cm), and increased with solution viscosity. An intriguing feature of previous experiments with ammonium chloride solutions is the formation of so-called 'chimneys'⁵. These are vertical channels in the mush through which strong upward flow of residual melt occurs, fed by lateral flow through the porous crystal network. Chimney formation was not observed in our experiments—we always observed the continuous generation of new starting plumes across the whole area of the tank. This is more consistent with the instability of a boundary layer at the top of the mush, analogous to the intermittent release of thermals from a diffusive boundary layer in high-Rayleigh-number thermal convection⁶. It may be that at higher solution viscosities chimneys develop only at times longer than the total duration of our experiments or that lateral flow in the mush is suppressed. Increasing the cooling rate at fixed viscosity resulted in a systematic increase of the critical time for the onset of convection, in agreement with previous qualitative observations^{5,7}. Furthermore, we have observed how the critical time varies with solution viscosity (Fig. 3b).

Theory shows that convective instability of a chemical boundary layer above a flat crystal face advancing at constant velocity^{8,9} occurs when a local Rayleigh number, defined using the boundary layer thickness (d) as a length scale, exceeds a critical

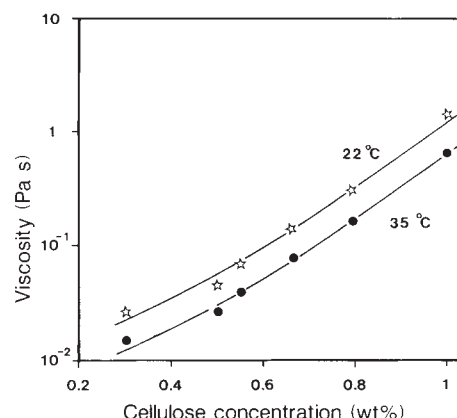


FIG. 1 The viscosity, determined with a falling-ball viscosimeter, of 28 wt% NH_4Cl solution as a function of the concentration of cellulose additive. The approximate initial temperature of the solution was 35 °C (lower curve), and the typical temperature measured at the crystallization front was 22 °C (upper curve). Viscosities from the latter curve were used in calculations on the instability of the compositional boundary layer.

value Ra_c given by

$$\frac{g\Delta\rho_c}{D\mu}d^3 = Ra_c \quad (1)$$

where $\Delta\rho_c$ is the compositional density difference across the boundary layer, g is the acceleration due to gravity, D is the diffusivity of the chemical species involved and μ is the solution viscosity. In this analysis, d is given by D/V , where V is the fixed rate of advance of the crystal face.

In our experiments, constitutional supercooling occurs and, instead of a flat crystal-liquid interface, a mush forms^{3,10}. Until the onset of compositional convection, theory predicts that temperature and composition evolve by diffusion in a regime for which the interface advances as $2\lambda\sqrt{Dt}$. The constant λ depends on the liquidus slope, the latent heat, the thermal and chemical diffusivities, and the cooling conditions^{2,3}. In this theory, crystallization starts instantaneously at time $t=0$, whereas in our experiments some time is required for nucleation and the establishment of a horizontal crystal layer (Fig. 2). Some non-zero value of initial time must be adopted in order to fit a $\sqrt{t}$ law, and times rather longer than the duration of our experiments are required to accurately verify this law. Depending on the initial temperature contrast, we obtain values of λ ranging from 3 to 6, which are close to those found using the theoretical framework of Worster³.

The compositional profile in the boundary layer above the mush is³:

$$C(z, t) = \Delta C \frac{\text{erfc}(z/2\sqrt{Dt})}{\text{erfc}(\lambda)} + C_i \quad (2)$$

where C_i is the initial liquid composition, ΔC is the compositional difference across the boundary layer and erfc is the complementary error function¹¹. Given the interface temperature and the assumption of local thermodynamic equilibrium, ΔC was estimated for this set of experiments to be ~ 0.1 wt% NH_4Cl . A rough check of this estimate was made by measuring the contrast in electrical conductivity of the solution across the boundary layer. The boundary layer thickness is defined as

$$d = \frac{\Delta C}{\left(\frac{\partial C}{\partial z}\right)} = \sqrt{\pi Dt} \exp(\lambda^2) \text{erfc}(\lambda) \quad (3)$$

which is of the form $d = \alpha(Dt)^{1/2}$. As $\lambda \rightarrow \infty$, equation (3) reduces to $d = D/V$, where the instantaneous velocity $V = \lambda(D/t)^{1/2}$. For $\lambda > 3$, as in our experiments, the difference between these expressions is less than 10%. Using the length scale D/V in equation (1) yields an expression for the critical time for the onset of convection, t_c :

$$t_c = \left(\frac{\lambda^3 Ra_c \mu}{g \Delta\rho_c \sqrt{D}} \right)^{2/3} \quad (4)$$

Our data support this viscosity scaling (Fig. 3b), which suggests that the essential physics of the situation is the same as for the case of a flat crystal face. Using $D = 2.3 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ (ref. 12) and $\Delta\rho_c = 1 \text{ kg m}^{-3}$, Ra_c lies between 1 and 8, with an average value of 3, which is at the lower end of the range of theoretical results for a flat crystal face⁹. This suggests that fluid from deep within the mush does not participate in the initial instability. Within this framework, plume diameter should scale with the thickness of the unstable boundary layer, which is also consistent with our data.

Thermal diffusion acts over a much greater thickness of fluid than merely the compositional boundary layer above the mush. The thermal field is such as to produce a stable density gradient but the compositional field is destabilizing. Theory predicts that until the onset of compositional convection, the temperature of

the mush-liquid interface is constant³. Figure 4 shows temperature profiles in the lower part of the tank, in two different experiments, recorded as the mush-liquid interface became level with each thermocouple in turn. The viscosities of the solution at the advancing interface were 1.4 Pa s (Fig. 4a) and 0.045 Pa s (Fig. 4b). In the first case (Fig. 4a) no convection was observed. The temperature of the mush-liquid interface is essentially constant, as expected. For long times, however, it exhibits a slight decrease, probably because our tank is of finite dimensions and hence the far-field temperature does not remain constant. In the latter case (Fig. 4b) compositional convection started after 27 min and continued throughout the experiment.

The main result is that a thick thermal boundary layer is observed despite the presence of compositional convection. The heat flux carried by the plumes is small, and manifests itself by slight quantitative differences in the evolution of the temperature profiles (Fig. 4) and the rates of advance of the mush-liquid interface in the convecting and conducting cases (Fig. 2). The plumes are thin and rise slowly, equilibrating with the surroundings by thermal diffusion over a time that is short compared with their rise time. Thus they are associated with very small temperature fluctuations ($< 0.05^\circ\text{C}$), as we have verified by a series of detailed continuous temperature measurements. This can be understood by considering the ratio of the buoyancy in the compositional boundary layer to that in the thermal boundary layer ($[\Delta\rho_c/\Delta\rho_T]\sqrt{D/\kappa}$, where κ is the thermal diffusivity of the solution). If this ratio has a value $\gg 1$, the plumes have enough buoyancy to thoroughly mix the thermal boundary layer into the overlying fluid. A ratio $\ll 1$ implies that the thermal stratification will be essentially undisturbed. The value of this ratio is ~ 0.05 in our experiments, and is ~ 0.005 for basaltic magmas. We conclude that, in this regime, the heat budget is dominated by conductive transfer.

This regime of compositional convection bears some resemblance to the 'salt-finger' regime of double-diffusive convection¹³. In salt-finger convection, the overall density gradient is stable and vertical motion is driven by horizontal diffusion of heat. Parcels of fluid retain their compositional difference with the surrounding fluid, but the vertical temperature gradient is unperturbed by the motion. In our experiments, the overall density gradient is unstable. Because the plumes are thin and sluggish, substantial lateral thermal diffusion occurs as they rise.

Our experiments illustrate that compositional convection involves two timescales, one characteristic of crystal growth and one characteristic of convective instability. Because the composi-

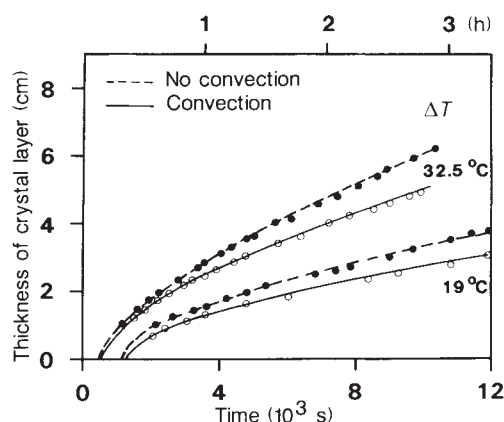


FIG. 2 The rate of growth of the crystal layer. ΔT is the temperature difference between the base plate and the initial temperature of the solution. Dashed curves are for experiments in which $\mu = 1.4 \text{ Pa s}$ and no convection occurred. Solid curves are for experiments in which $\mu = 0.045 \text{ Pa s}$ and abundant convection occurred. Each pair of a dashed and a solid curve show the difference in the advance of the interface for experiments with the same thermal conditions. Differences are small, indicating that compositional convection makes a small contribution to the heat budget.

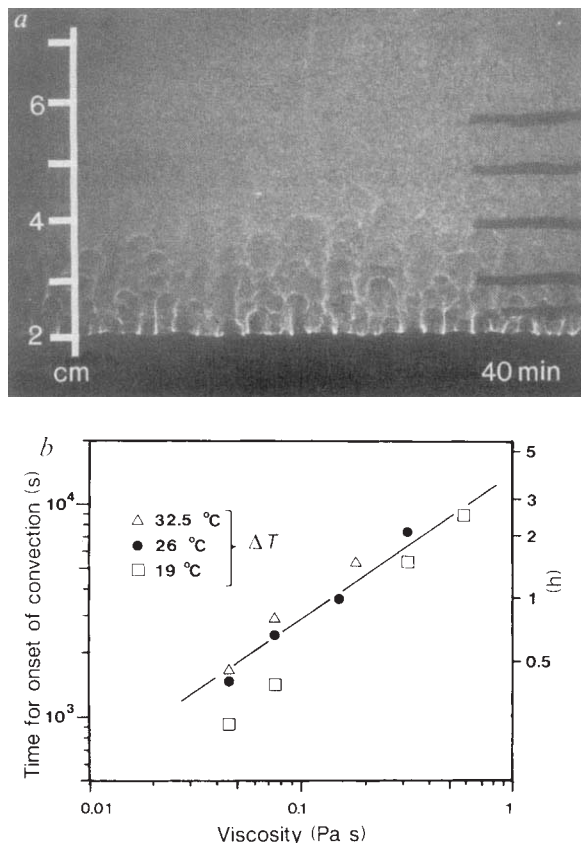


FIG. 3 *a*, Photograph showing the development of crystallization and compositional convection at the base of the tank ($\mu = 0.045$ Pa s). *b*, Measurements of the critical time (t_c) for the onset of convection as a function of solution viscosity. ΔT is the temperature difference between the base plate and the initial temperature of the solution. t_c increases systematically with cooling rate. The data are also consistent with the prediction based on a local Rayleigh-number instability criterion for the boundary layer that t_c scales with solution viscosity to the power $2/3$ (solid line). This line is drawn for $Ra_c = 3$.

tional boundary layer itself develops by diffusion during crystal growth, it is not possible for the latter to be much shorter than the former. Approximate equality of the two timescales corresponds to the case of aqueous solutions. The other possibility, crystal growth being more rapid than the development of convective instability, may arise naturally in, for example, igneous intrusions.

In mafic magma, growth of olivine and pyroxene releases less dense residual melt and thus, by analogy with our experiments, compositional convection can occur. At the onset of convection, the vertical flux into the crystallizing region of the compatible chemical components is increased, which should result in an increase in the modal proportions of these minerals in the crystallized product. Although large undercoolings can be attained, the propagation of the crystallization front during crystallization of magma always occurs at a rate proportional to $\sqrt{t}$ (ref. 14). We assume that compositional convection begins when the local solutal Rayleigh number exceeds a value of 3. From equation (1), the critical value of the mush-liquid interface velocity below which convection can occur, V_c , is

$$V_c = \left(\frac{g \Delta \rho_c D^2}{\mu Ra_c} \right)^{1/3} \quad (5)$$

For an illustrative calculation for basaltic magmas, we take $\mu = 10$ Pa s, $D = 10^{-12}$ m² s⁻¹ (values corresponding to Mg²⁺ and Fe²⁺ in mafic melts¹⁵) and $\Delta \rho_c = 10$ kg m⁻³ (ref. 16), for which equation (5) gives $V_c = 1.5 \times 10^{-8}$ m s⁻¹, that is, a few tens of centimetres per year.

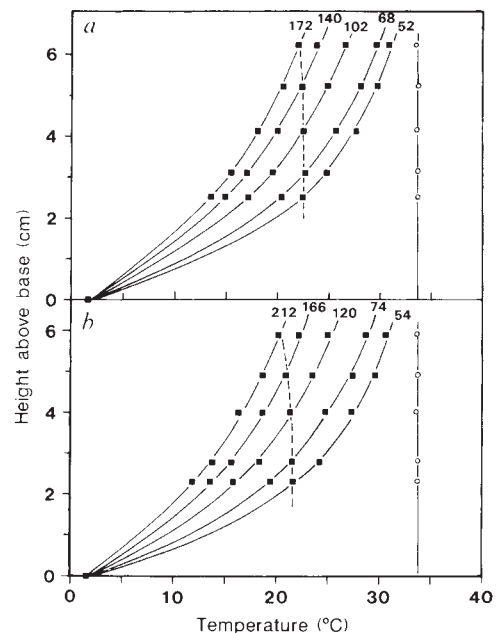


FIG. 4 Vertical profiles of temperature in the lower part of the tank, revealing the structure of the double-diffusive boundary layer generated by crystallization. Open circles show the initial uniform temperature of the solutions. The numbers give the time in minutes at which profiles were recorded. Dashed lines show the crystallization-front temperature, solid line segments to the right of the dashed lines show the profiles above the front, and those to the left show the profiles in the mush. In both experiments, the solution has a liquidus temperature of 24 °C and the base plate temperature was 1.5 °C. *a*, $\mu = 1.4$ Pa s, $\Delta T = 32.5$ °C. No convection occurred, and temperature evolved by conduction alone. *b*, $\mu = 0.045$ Pa s, $\Delta T = 32.5$ °C. Despite abundant compositional convection, there is a thick thermal boundary layer above the front—see discussion in text.

A question of practical importance to petrologists is: what thickness of rock will have crystallized by the time compositional convection starts? The crystallization front $X(t)$ moves according to $X = 2\lambda^* \sqrt{\kappa t}$ (ref. 14) and thus the front velocity is $V = \lambda^* \sqrt{\kappa/t}$. Hence

$$X(t) = 2\lambda^* \kappa / V \quad (6)$$

Using the above value of critical velocity and taking $\kappa = 7 \times 10^{-7}$ m² s⁻¹ and $\lambda^* = 1$ (ref. 14), we find that ~ 90 m of rock would crystallize before the onset of convection. Clearly there is a range in the values of the governing parameters for natural magmas, and here we intend to obtain only an order-of-magnitude estimate.

In mafic sills, a layer with increased modal olivine content is commonly observed at a certain distance from the lower contact. The height above the lower contact of olivine concentrations for several sills of different overall thicknesses and related to different magmatic episodes ranges from 10 to 20 m (refs 17–20). In large mafic intrusions, there is typically a basal sequence overlain by thick olivine cumulates. In the Stillwater Complex, North America²¹, the Muskox Intrusion, Canada²² and the Great 'Dyke', Zimbabwe²³, these basal sequences are observed to be about 100 m thick. The above thicknesses are all of roughly the same order of magnitude, suggesting that the appearance of mineralogical layering results from local phenomena. We propose that this may be due to the onset of compositional convection, because the thicknesses are broadly similar to the result obtained above from equation (6). This hypothesis can be verified by further petrological studies. □

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Contamination of Indian Ocean asthenosphere by the Kerguelen–Heard mantle plume

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THE mid-ocean-ridge basalts (MORBs) from the Indian Ocean are isotopically distinctive. They have higher $^{87}\text{Sr}/^{86}\text{Sr}$, $^{208}\text{Pb}/^{206}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ ratios than their Pacific and Atlantic counterparts^{1,2} (see Fig. 1), suggesting that the upper mantle beneath the Indian Ocean has remained a partially isolated system². The Australian–Antarctic discordance, a bathymetrically depressed segment of the South-East Indian Ridge, may represent a fundamental boundary between the Indian and Pacific mantle domains³. What processes are responsible for such domains? We argue here that the Kerguelen–Heard plume, which is producing Dupal-type basalts on Kerguelen and Heard Islands in the southern Indian Ocean, has contaminated large volumes of the Indian Ocean asthenosphere. This has produced the distinctive composition of Indian Ocean MORBs.

Figure 2 illustrates the tectonic evolution of the southern Indian Ocean, and the development of tectono-magmatic features that may be attributable to the Kerguelen–Heard plume. The diagrams suggest that the plume has been involved in the development of several major features of the Indian Ocean lithosphere, and indicate that it has been active during the past 115 Myr. Lower Cretaceous continental magmatism in south-west Australia (Bunbury Basalts: 105–136 Myr BP⁴), north-east India (Rajmahal Traps: 108–128 Myr⁵ and lamprophyres: 105–121 Myr⁶) and Antarctica (Prince Charles Mountains lamprophyres: 108–110 Myr⁷) imply that the plume was active during, and perhaps before, the separation of India from Antarctica–Australia at ~122 Myr⁸.

Major oceanic structures that can be attributed to the Kerguelen–Heard plume are the Kerguelen Plateau, emplaced during the Lower Cretaceous⁹, possibly at a ridge setting

analogous to that of Iceland today; and the Ninetyeast Ridge, a 5,000-km-long aseismic ridge¹⁰. Both features testify to huge volumes of magma being erupted throughout much of the Cretaceous and Tertiary. But do the chemical data for these features support the contention that they were all derived from the Kerguelen–Heard Plume? And do the data support the hypothesis that the same plume has contaminated large volumes of the Indian Ocean asthenosphere?

Kerguelen Island has been magmatically active for at least the past 40 Myr¹¹. An important feature of Kerguelen Island magmatic activity is the changing isotopic and trace-element compositions with time; the younger basalts have higher ϵ_{Sr} and lower ϵ_{Nd} (refs 12, 13). During its early history, the island (and the hotspot) was probably adjacent to the embryonic South-East Indian Ridge. Mixing between plume mantle and either MORB asthenosphere or sub-plateau lithosphere diluted the effect of the plume¹². Thus, the younger basalts may be isotopically more representative of the plume mantle, for example: $^{87}\text{Sr}/^{86}\text{Sr} \approx 0.7051$ – 0.7059 ; $\epsilon_{\text{Nd}} \approx -1.7$ to -5.7 ; $^{208}\text{Pb}/^{204}\text{Pb} \approx 38.5$. These isotope data are typical of Dupal-type oceanic basalts¹⁴.

Basalts from Kerguelen and Heard Islands form an array with approximately constant La/Th ratios (Fig. 3). The elements La, Ta and Th are highly incompatible in basaltic systems and ratios of these elements probably reflect the compositions of the mantle source; certainly, most ocean islands plot in distinct fields on this diagram¹⁵. Such systematic variations cannot be easily accommodated by fractionation during magma genesis. The Kerguelen and Heard array overlaps the field for Gough Island, an island in the South Atlantic with a strong Dupal component¹⁶, although some Heard Island samples have lower Th/Ta and La/Ta ratios.

In Pb-isotope space, two analysed tholeiites from the Ninetyeast Ridge¹ overlap the field of Kerguelen–Heard basalts (Fig. 1), suggesting a Dupal-like character. In terms of certain trace-element ratios (for example, Zr/Nb ratios¹⁷), the tholeiites are intermediate between Kerguelen Island plume basalts and MORBs. There is considerable scatter on the Th/Ta–La/Ta diagram, however, and many of the samples do not fall on such a mixing line (Fig. 3).

A suite of dredged tholeiites from the 77° graben⁹, in the southern part of the Kerguelen Plateau, has been analysed for Pb, Sr and Nd isotopes and major and trace elements. These results will be published elsewhere. The basalts are characterized by high $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ ratios, overlapping with the data from the Ninetyeast Ridge, the South-East Indian Ridge

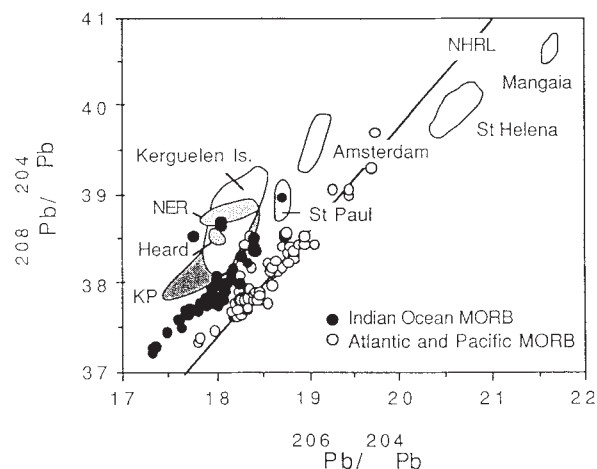


FIG. 1 $^{208}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ for Indian, Atlantic and Pacific oceanic basalts. Data sources: Atlantic and Pacific MORB, refs 24–26; Indian MORB, refs 24–27 (and references in ref. 12); Kerguelen Plateau (KP), ref. 18; Kerguelen and Heard Island, ref. 12; Ninetyeast Ridge (NER), ref. 1; St Paul, refs 1 and 2; Amsterdam, ref. 1; St Helena, ref. 16; Mangaia, ref. 29; Northern Hemisphere Reference Line (NHRL), ref. 14.

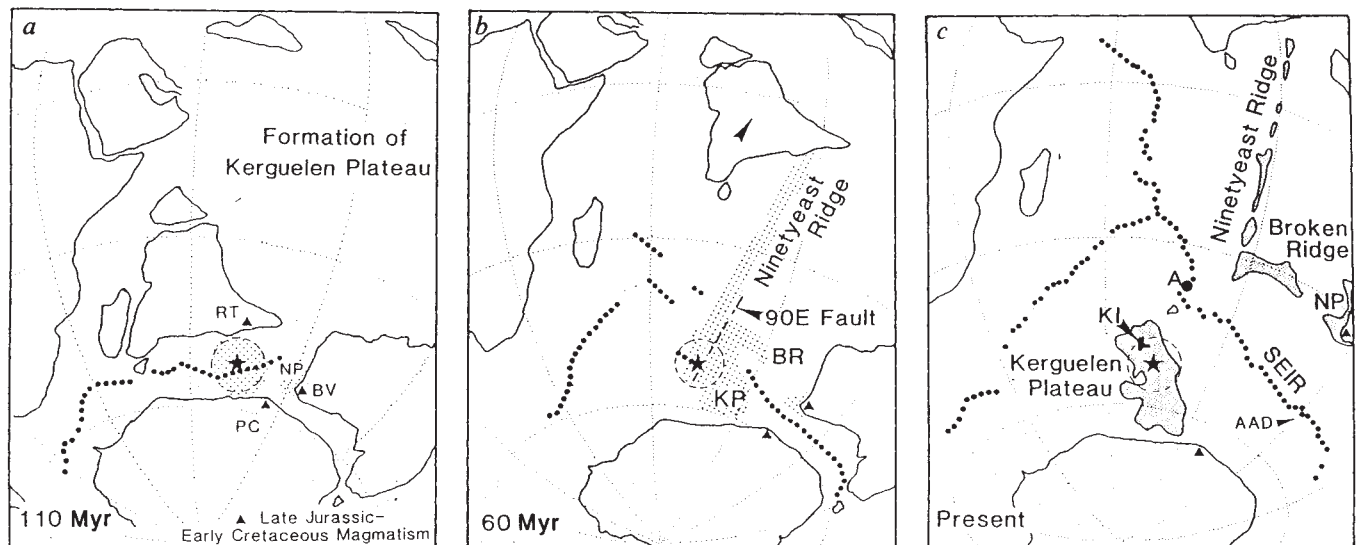


FIG. 2 Schematic maps to illustrate the evolution of the southern Indian Ocean, and the interactions of the Kerguelen-Heard plume (KHP) with various plate boundaries. The reconstructions were created using the computer package 'Terra Mobilis' (C. Denham & C. Scotese, Geoimages, Texas). The plume (with a 1,000-km-diameter head) is assumed to have a fixed coordinate beneath the present-day location of Heard Island (star). KI, Kerguelen

Island. BV, Bunbury Volcanics (ref. 4); RT, Rajmahal Traps and lamprophyres (refs 5 and 6); NP, Naturaliste Plateau; PC, Prince Charles Mountains lamprophyres (ref. 7); SEIR, South-East Indian Ridge; AAD, Australia-Antarctic Discordance (ref. 3); A, Amsterdam and St Paul Islands. Stippled ornament represents regions of thickened oceanic lithosphere.

MORBs, and the Kerguelen Archipelago (Fig. 1)¹⁸. A striking feature of the Kerguelen Plateau data is the range of Th/Ta and La/Ta ratios of the dredged basalts (Fig. 3), perhaps indicating an extreme expression of the Dupal component observed in Kerguelen Island basalts¹². The extent to which the high Th/Ta ratios observed in the plateau basalts are representative of the plateau as a whole must await further data from basalts recovered during Leg 120 of the Ocean Drilling Program. We note here that high Th/Ta ratios are found in some continental flood basalts¹⁹ and lamprophyres²⁰ (Fig. 3), and they are probably a feature of the continental lithosphere (crust or sub-continental mantle). The high Th/Ta ratios found in four analysed Kerguelen Plateau basalts cannot be taken to indicate that the plateau is underlain by subcontinental lithosphere. However, one interpretation is that the sub-Gondwana lithosphere, with high Th/Ta ratios, was activated and stripped by the Kerguelen-Heard plume and incorporated in the sub-Gondwana asthenosphere (Fig. 4), and then melted out during the formation of the Kerguelen Plateau lithosphere. Alteration of the basalts to produce high Th/Ta and La/Ta ratios cannot be completely excluded but we note that all three elements have low mobility during the low-temperature alteration which has affected these samples.

The possibility arises that the long-lived, vigorous Dupal Kerguelen-Heard plume is responsible for the Dupal-like character of modern Indian Ocean MORBs, through pervasive contamination of the sub-oceanic asthenosphere. Hamelin *et al.*² have suggested that mixing between MORB asthenosphere and plume mantle is responsible for generating the distinctive Indian MORB isotopic arrays. To fit the data, at least two stages of mixing are required: (1) an earlier contaminant (the Kerguelen-Heard plume in this paper) which was responsible for the broad elevation in $^{208}\text{Pb}/^{206}\text{Pb}$ (and $^{87}\text{Sr}/^{86}\text{Sr}$) ratios, and which affected large volumes of the Indian Ocean asthenosphere; and (2) subsequent mixing between this contaminated asthenosphere and other high- $^{206}\text{Pb}/^{204}\text{Pb}$ mantle plumes in the central Indian Ocean (for example, St Paul and Amsterdam islands). This second event appears to be responsible for the spread to high $^{206}\text{Pb}/^{204}\text{Pb}$ values on Pb-Pb isotope plots (Fig. 1). The La/Ta-Th/Ta data are broadly consistent with mixing between depleted MORB mantle (DMM), with a high

La/Ta ratio¹⁵, and the plume mantle. (Unfortunately, no suitable trace-element data are available for basalts from St Paul and Amsterdam islands.) However, none of the analysed South-East Indian Ridge basalts has high Th/Ta ratios. This suggests that mixing between a DMM and a high Th/Ta (lithospheric?) component was not an important process in the evolution of the sub-Indian Ocean MORB asthenosphere, although Th, La and Ta data are required from other Indian Ocean ridge segments to confirm this suggestion.

Evidence for plume contamination of adjacent sub-oceanic asthenosphere is preserved elsewhere; for example, Iceland²¹,

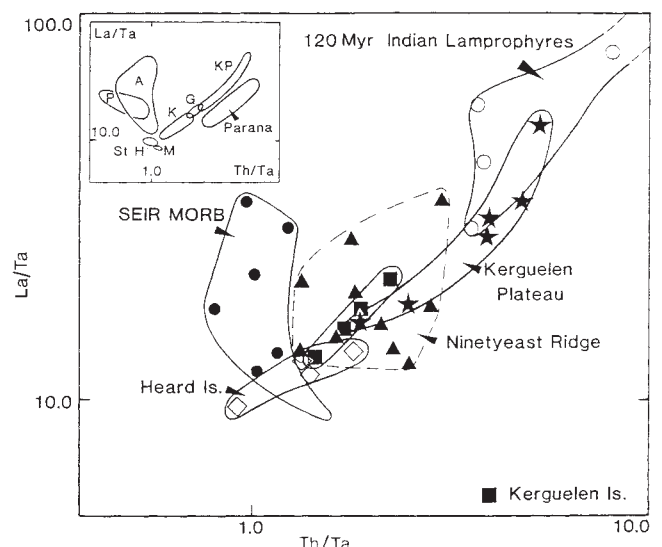


FIG. 3 La/Ta against Th/Ta for Indian Ocean basalts and (inset) other oceanic and continental (Parana) regions. Data sources: Heard and Kerguelen (K) Islands, ref. 12; SEIR MORB, ref. 30; Ninetyeast Ridge (DSDP Sites 214, 216, 253 and 254) and Kerguelen Plateau (KP), this study; Indian lamprophyres, ref. 20; Pacific (P) and Atlantic (A) MORBs are extrapolated from diagrams in ref. 15 (and references therein) assuming a Ce/La ratio of 3.0 and a Nb/Ta ratio of 17.65; Mangaia (M), ref. 28; Gough (G) and St Helena (St H), ref. 16; Parana continental flood basalts, ref. 19.

Easter²² and Ascension²³ islands. However, in these examples the extent of contamination appears to be far less widespread than in the Indian Ocean. There may be several reasons for this. First, the Indian Ocean is a relatively compact, youthful ocean, and it originated from a locus at the Kerguelen–Heard plume. Second, the plume is very distinct from MORB in having a strong Dupal character. The component is thus more easily detected in the MORBs than are the Icelandic and Ascension signatures. Third, the plume has interacted with major spreading axes throughout its history, and therefore has had access to the Indian Ocean mantle system (the other main Dupal islands, Gough and Tristan da Cunha in the South Atlantic, have had relatively localized access to the Atlantic system); and finally, the plume appears to have been active for a long period of time, and there is strong evidence (mentioned above) that it reactivated the sub-continental lithosphere above the embryonic Indian Ocean asthenosphere. The plume could therefore have been actively contaminating the asthenosphere before the break-up of Gondwana.

Identification of mantle domains associated with specific mantle plumes can only be achieved by detailed dredging of the present-day ridge system. Klein *et al.*³ have recently identified a potential boundary between two oceanic mantle domains,

which may represent the eastern influence of the Kerguelen plume system. There are too few data to identify the western limit of the influence of the KHP; the basalts of the South-West Indian Ridge appear to have a Walvis Ridge component², indicating the influence of another Southern Hemisphere Dupal domain. The unknown factor is the extent to which many of the distinctive Dupal characteristics—in plumes or MORBs—result from long-term residence beneath the stable Gondwana supercontinent.

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Genetic specialists, kin recognition and nepotism in honey-bee colonies

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INSECT societies have long served as useful models in the study of two often intertwined issues in evolutionary biology: the levels at which natural selection operates¹, and the phenomena of competition and cooperation in animal societies². Hamilton^{3–5} provided a darwinian explanation for the evolution of cooperation by invoking natural selection for increased inclusive fitness, whereas Trivers and Hare⁶ showed how asymmetrical genetic relationships among members of hymenopteran societies result in differential fitness among queens and workers and can lead to conflict over the production of males. Here we provide evidence for competition among workers of the honey bee, *Apis mellifera*, in the production of female reproductives, a consequence of the genetic structure of colonies resulting from polyandry⁷, genotypic biases in components of cooperative behaviour associated with division of labour^{8–10}, and kin recognition^{11–13}. We also propose that nepotistic behaviour by honey bees is influenced by both kin- and colony-level selection.

Honey-bee colonies normally consist of about 17 subfamilies⁷. Members of the same subfamily, super sisters¹⁴, share both a

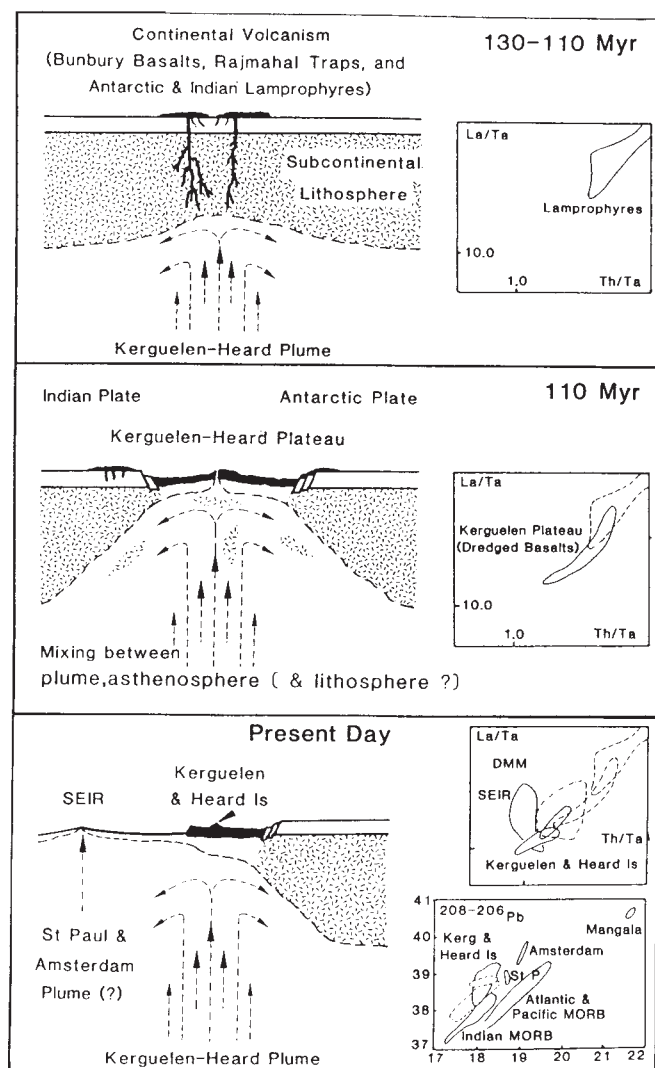


FIG. 4 Schematic diagrams to illustrate the interaction of the Kerguelen–Heard Plume with the sub-Gondwana lithosphere and with the sub-Indian Ocean asthenosphere. See text and Figs. 1–3 for discussion and data sources. DMM: Depleted MORB mantle (see ref. 15).

queen mother and a drone father and, in the absence of inbreeding, have a coefficient of genetic relationship of 0.75 (ref. 15). Half-sisters belong to different subfamilies, share only a mother, and have a genetic relationship of 0.25. We tested the ability of workers to discriminate during the rearing of queens in colonies that were derived from instrumentally inseminated queens and composed of three biochemically-distinct subfamilies. Samples of immature (larvae and prepupae) queens and workers, and adult workers located near either immature queens or workers, were collected from experimental colonies and analysed by protein electrophoresis to determine the frequencies of individuals belonging to each of the three subfamilies (see Fig. 1 and Table 1 for details).

Any observed subfamily biases in queen production could be a consequence of intracolony kin recognition coupled with one or more of four mechanisms: (1) workers preferentially raise the most familiar larvae, that is, those belonging to the subfamily with the highest larval frequency; (2) workers prefer-

entially raise the rarest larvae; (3) the subfamily with the most adult members on queen cells dominates queen rearing and preferentially raises super sisters; and (4) biases occur in the proportional representation of subfamilies engaged in the rearing of queens (relative to larval frequencies) and individuals preferentially rear super sisters. To determine which, if any, of these mechanisms are involved, four series of Monte Carlo simulations were conducted based on the results of our allozyme analyses. Each series of simulations focused on a different 'test'-subfamily that was appropriate to each one of these four possible mechanisms.

Our results demonstrate nepotism during the rearing of queens. Individual workers were located on or off queen cells, at least in part, on the basis of genotypically determined factors. Treatment of immature queens (involving perhaps differential feeding and/or selective removal) was apparently mediated by genetic relatedness with a small but consistent rearing preference shown for more closely related larvae. Subfamilies with a bias

TABLE 1 Nepotism in the rearing of queens by honey bees

Test-subfamily						Test-subfamily					
Total no. immature workers analysed	Total no. queens analysed	Weighted average of frequency in immature worker and queen samples	Expected no. queens reared	Observed no. queens reared	Deviation	Total no. immature workers analysed	Total no. queens analysed	Weighted average of frequency in immature worker and queen samples	Expected no. queens reared	Observed no. queens reared	Deviation
Colony 4438						Colony 4450					
49	29	0.46	12	15	+3	40	32	0.07	0	5	+5
50	28	0.37	10	11	+1	40	38	0.35	11	15	+4
39	7	0.33	2	3	+1	40	14	0.00	0	0	0
Colony 4439						Colony 4453					
40	6	0.35	2	3	+1	40	25	0.45	10	12	+2
40	24	0.38	10	8	-2	40	26	0.24	6	6	0
40	7	0.26	2	0	-2	40	17	0.28	5	3	-2
Colony 4440						Colony 4456					
50	33	0.30	9	11	+2	49	32	0.19	6	5	-1
41	44	0.39	13	21	+8	51	44	0.40	15	20	+5
40	28	0.31	8	9	+1	40	37	0.41	14	17	+3
Colony 4442						Colony 4457					
40	31	0.39	11	6	-5	40	36	0.38	10	18	+8
40	40	0.40	14	18	+4	40	40	0.55	19	25	+6
40	50	0.10	2	7	+5	40	53	0.28	15	15	0
Colony 4445						Colony 4464					
50	35	0.29	8	13	+5	40	29	0.06	1	2	+1
50	41	0.13	2	9	+7	40	36	0.04	1	2	+1
41	25	0.23	6	6	0	34	32	0.64	21	20	-1
Total deviation = +60											

The table demonstrates nepotism in the rearing of queens by honey bees based on kin recognition and subfamily differences in the likelihood of raising queens (mechanism 4 in text). In this case the 'test'-subfamily (one per trial for each colony, three trials total) was selected because it exhibited a higher probability of being located on queen cells than other subfamilies in the colony. For the analysis, four different sets of 'test'-subfamilies were selected, each set appropriate to one of the four hypothesized mechanisms underlying biases in the rearing of queens. To test the hypothesis of mechanism 4 we determined the relative likelihood of rearing queens, RL , for individuals of each subfamily (s) within a colony: $RL_s = PA_s/PI_s$, where PA_s is the proportion of subfamily s in sample of adults on queen cells, and PI_s is the proportion of subfamily s in sample of immature workers. The subfamily with the highest value of RL for each colony in each trial was designated the 'test'-subfamily for mechanism 4. The expected number of queens for the test-subfamily $E(Q_t)$ was then calculated from the equation $E(Q_t) = (I_t/N)$, where I_t is the proportion of test-subfamily in sample of immature workers, and N is the total number of queens raised in a given trial. The difference between $E(Q_t)$ and the observed number of queens $O(Q_t)$ was then calculated to determine the deviation. Because conventional analyses of contingency tables were inappropriate¹⁶ (workers selected queens without replacement from among a finite sample of 60 transferred larvae), we used Monte Carlo simulations to estimate the probability of getting a deviation that was as great or greater than our result. For each of the four hypothesized mechanisms, the Monte Carlo simulation randomly drew a number of immature workers equal to the actual sample from a very large (infinite) pool with appropriate test-subfamily frequencies equal to the weighted average of the immature worker and queen samples. It then drew an independent random sample of immature queens from the same pool and calculated the deviation from expected as described above. Deviations were summed for all trials of all colonies. The simulation was iterated 1,000 times and iterations in which the total deviation was as great or greater than that observed were summed. The probability of getting a result as extreme as that observed was calculated as the number of iterations with a total deviation greater than that observed divided by 1,000. The entire simulation process was repeated five times to give an estimate of the standard error of the simulation-generated probability. Observed deviations cannot be a consequence of nonrandom sperm usage by queens because $E(Q_t)$ and $O(Q_t)$ were both based on the same samples of immature females.

in adult representation as queen nurses, relative to the available potential queen larvae, biased the colony's output of queens in their favour.

On average, only half ($\bar{X} = 30 \pm 11.8$ s.d.; 916 queens were raised in total) of the 60 transferred larvae were raised during each trial of each colony, thus providing ample opportunity for workers to make 'choices' with respect to which larvae become queens. Combining data from all trials of all colonies, the subfamilies whose adult members had the highest within-trial individual probability of being sampled on queen cells (mechanism 4; Fig. 1) collectively had 60 more super-sister queens raised than expected on the basis of samples of immature workers ($P = 0.001 \pm 0.0002$ s.e., $n = 5$ Monte Carlo simulations; see Table 1). In contrast, members of the numerically most common subfamily, again based on samples of immature workers (mechanism 1), had 37 fewer queens raised than expected. Individuals belonging to the rare subfamily (mechanism 2) had 13 more than expected ($P = 0.179 \pm 0.0066$), and those belonging

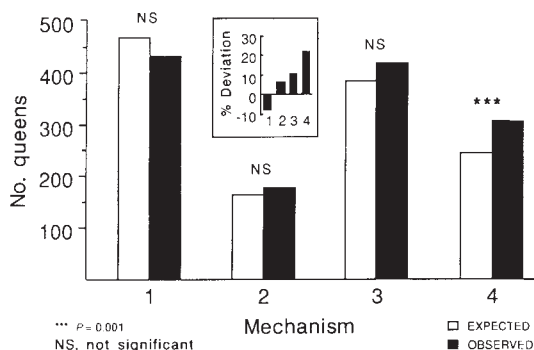


FIG. 1. Expected and observed number of queens reared for each of four mechanisms hypothesized to result (along with kin recognition) in nepotism in the rearing of queens by honey bees. The inset depicts the per cent deviation of the observed from the expected values for each mechanism. For explanation of statistical analyses see legend for Table 1.

METHODS. Ten colonies were established, each composed of three electrophoretically distinguishable subfamilies. The colonies' queens were daughters of three unrelated queen mothers. Drones for matings were progeny of eight additional unrelated queens. Each of the ten queen daughters was instrumentally inseminated²³ with semen from three unrelated drones, each one bearing a different malate dehydrogenase-1 (*Mdh*) marker allele, designated 'slow' (S), 'medium' (M) and 'fast' (F)²⁴. Semen from each drone trio was pooled, diluted, and mixed before insemination to minimize temporal fluctuations in subfamily frequencies^{25,26}. Four of ten inseminated queens were homozygotes at the *Mdh* locus (SS: colonies 4438, 4440, and 4456; MM: colony 4453), producing female progeny with three allozyme phenotypes that correspond to three subfamilies. Five queens (colonies 4439, 4442, 4445, 4450, and 4464) were SF heterozygotes that produced three subfamilies with five allozyme phenotypes: SS, SM, SF, MF, and FF. Larval and adult bees sampled from these colonies were grouped into three subfamilies as follows. SM and MF bees were assigned to the M subfamily because the M allele could only be paternally inherited. SF bees were assigned to the S and F subfamilies in proportion to the ratio of SS and FF phenotypes in each sample, because the maternal S and F alleles are expected to segregate in a 1:1 ratio. Subfamily membership was determined similarly for individuals from colony 4457, derived from a SM queen. The experiment took place from April to May, 1987, under conditions of seasonal production of queens associated with normal colony reproduction through fission²⁷. At the start, nine of ten colonies (all except 4445) were raising queens naturally. Colonies occupied hives consisting of two or three chambers; the lower two were filled with combs of brood and honey, and the third with honey and empty combs. Each queen was confined to a bottom chamber of the hive with a queen excluder. For each colony, 60 newly hatched female larvae were transferred from a frame of worker comb containing up to 3,700 individual larvae into dry, artificial queen cells arranged 20 cells to a bar, three bars to a frame (female larvae located in special queen cells are fed differentially and develop into queens rather than workers²⁸). The two frames were placed together in the centre of the upper brood chamber in each hive, with the queen cells closest to the side from which larvae were transferred. Three days later we removed the two frames, with minimal disturbance, and collected ~50 workers each from the surface of the queen cells and from the area of worker comb that originally contained the transferred larvae. These were assumed to be queen and worker 'nurses', respectively. Immatures (larvae and prepupae) were sampled six days after transfers when all investment in new queens was complete and the queen cells were capped by the workers²⁸. In addition to collecting all queen cells, we gathered samples of about 50 immature workers that were the same age and located in the same area of the comb as were the queen larvae before they were transferred. All samples were stored at -70°C until analysed by electrophoresis. Each colony was tested three times at 7–8-day intervals for a total of 30 trials. All queens, 34–51 immature workers, and 34–43 adult workers per sample were analysed.

to the subfamily with the highest frequency of adult workers on queen cells, regardless of numerical representation in the colony (mechanism 3), had an excess of 34 raised ($P = 0.064 \pm 0.0034$). In 13 of the 18 trials that showed a positive bias in the rearing of queens, assuming mechanism 3, the numerically dominant adult subfamily was also the one whose members had the greatest bias in likelihood of being sampled as queen nurses and, therefore, were also classified as the test subfamily based on mechanism 4. In these cases, mechanisms 3 and 4 were confounding.

Genetic 'specialization' for rearing queens is suggested by the following results. In four of ten colonies, the subfamily distribution of adult workers sampled on queen cells differed significantly from the distribution of adult workers sampled on the adjacent comb containing worker larvae in at least one of three trials, even though the sampled frames were only ~2–4 cm apart ($P < 0.05$, colony 4440; $P < 0.01$, 4450; $P < 0.01$, 4456; $P < 0.05$ and $P < 0.01$, 4464; G tests of heterogeneity¹⁶). In two colonies subfamily differences were significant for the three trials combined ($P < 0.01$, 4453 and $P < 0.05$, 4456). The number of trials that showed a significant deviation ($P < 0.05$) was greater than that expected based on chance alone ($P < 0.02$; binomial test, assuming 30 independent trials). These differences may be due either to paternally inherited behavioural traits that generate a bias in the representation of adult subfamily members on queen cells relative to their representation in the pool of potential queen zygotes, or, alternatively, to nonrandom sperm use by queens coupled with age differences between queen 'nurses' and worker 'nurses'. Results based on analyses of patterns of sperm use for each queen do not support the alternative explanation. The subfamily distribution in worker larvae samples fluctuated significantly among trials in only two of ten colonies ($P < 0.01$, 4445 and 4450), suggesting that the sperm of the three drones used for each insemination were well mixed in eight colonies.

Subfamily biases for the rearing of queens were small and consistent with previous studies. The ratio of the proportion of test-subfamily (assuming mechanism 4) adults sampled on queen cells to its proportion in the sample of worker larvae (see Table 1 legend) was 2.3 ± 1.90 s.d. The mean ratio of the observed to the expected number of test-subfamily (mechanism 4) queens raised, based on the worker larval samples, was 1.5 ± 1.10 s.d. Page and Erickson¹⁷ reported a ratio of 1.6 in the number of queens reared when workers were given a choice of super-sister and less related ($G = 0.25$ or 0.31) non-nestmate larvae. Visscher¹⁸ found a bias of 1.5 in favour of a mixture of super- and half-sister nestmate eggs or larvae over unrelated non-nestmates. Noonan¹⁹ demonstrated a bias of 1.2 in the visitation frequency of adult workers to queen cells containing super- versus half-sister larvae in colonies composed of two visually distinguishable subfamilies.

In agreement with previous studies^{8–10}, our results suggest that the genotypes of workers affect colony organization by influencing the probability of performing behaviour associated with the division of labour. Worker genotype also results in differential social interactions, some of which may lead to intranidal conflict. Simply determining genetic relationships among colony members is not sufficient for testing kin-selection theory; the genetic relationships among interacting individuals must be considered (compare with ref. 20). Our results also demonstrate that kin selection is an important process in the evolution of social interactions involved in reproduction.

Larval kin recognition should increase the adaptive value of genotypes that result in a higher probability of the rearing of queens, driving them rapidly to fixation in populations. Perhaps this is a social analogue of meiotic drive²¹ that distorts the proportional representation of queen genotypes (M.D. Breed, personal communication). Genetic variability and the rather modest levels of nepotism observed may be functionally linked and represent a compromise between levels of selection²². Kin selection may favour alleles that result in intracolony competition: high propensity to rear queens, better recognition, and

more discrimination. However, variation in the rearing of queens coupled with larval discrimination may reduce the reproductive output of colonies, a consequence of decreased rearing of queens due to adult-adult or adult-larva conflict, or a decrease in the ergonomic efficiency of the colony. If so, then colony-level selection may favour cooperation: a lower propensity for rearing of queens, and less discrimination. With additional genetic analyses of insect societies, the functional significance of the observed genetic variability, and the mechanisms underlying the intricate interplay of competition and cooperation may be further elucidated. □

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Root lectin as a determinant of host-plant specificity in the *Rhizobium*-legume symbiosis

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THE induction of nitrogen-fixing nodules in legume roots by soil bacteria from the genera *Rhizobium* and *Bradyrhizobium* is host-plant-specific. This specificity is expressed at an early stage of the infection process and results from multiple interactions between bacterial and plant products. Among these, it has been suggested that root lectin recognized by bacterial receptor molecules is an important determinant of host specificity^{1,2}. Lectins are carbohydrate-binding proteins and it is known that legumes belonging to different cross-inoculation groups produce lectins with different sugar-binding specificities³. We have tested this suggestion by introducing the pea lectin gene into white clover roots using *Agrobacterium rhizogenes* as a vector. The 'hairy' clover roots that result can be nodulated by a *Rhizobium* strain usually specific for plants from the pea cross-inoculation group.

Rhizobium leguminosarum bv. *viciae* nodulates the cross-inoculation group of pea, vetch and lentil, whereas clover is nodulated by *R. leguminosarum* bv. *trifolii*. The involvement of root lectin in determining this host-plant specificity was tested

by introducing the pea lectin gene (*psl*) into the roots of white clover and by studying the infection of transgenic clover roots by *R. leguminosarum* bv. *viciae*. Transgenic roots rather than transgenic plants were studied because of the problems of regenerating the latter.

We cloned *psl* into a binary vector⁴ using *A. rhizogenes* LBA1334 (Ar1334) (ref. 5) to obtain transgenic clover roots. The *psl* gene encodes a glucose/mannose binding protein^{6,7} and is expressed at low and developmentally controlled levels in pea roots during the plant's life cycle⁸. Pea lectin is localized at the surface of growing pea root hairs, which are the target cells for rhizobial infection⁹. Lectin-enhanced accumulation of infective rhizobia at pea root-hair tips correlates with increased infectivity of the rhizobial cells¹⁰.

Complete genomic *psl* (*psl* lecA provided by J. A. Gatehouse⁷) was cloned in the binary vector pBin19 (ref. 11). In addition, the full length *psl* complementary DNA¹² (provided by M. Stubbs) was cloned in the binary expression vector pAGS 35S HB (ref. 13) (provided by C. M. P. van Dun) between the plant viral 35S promoter and the nopaline synthase 3' end. These binary vectors are referred to as pBin19 *psl* and pAGS *cpsl*, respectively. (For details of the cloning steps see Fig. 1.)

The efficiencies of the binary vector systems were tested by determining the activity of a reporter gene product. Both vectors carry a neomycin phosphotransferase gene (NPTII, see Fig. 1) which can be expressed in transformed plant tissue. Hairy roots

TABLE 1 Nodulation of white clover hairy roots by *Rhizobium leguminosarum*

Agrobacterium strain and binary vector	No. nodulated plants			
	Inoculation with bv. <i>trifolii</i>			
	*10 d	23 d	30 d	40 d
LBA1334	13/20	18/20	20/20	20/20†
LBA1334 pBin19	7/15	14/15	15/15	15/15†
LBA1334 pBin19 <i>psl</i>	15/20	19/20	20/20	20/20†
LBA1334 pAGS <i>cpsl</i>	15/19	19/19	19/19	19/19†
	Inoculation with bv. <i>viciae</i>			
LBA1334	0/19	0/19	0/19	1/19‡
LBA1334 pBin19	0/15	0/15	0/15	0/15
LBA1334 pBin19 <i>psl</i>	0/20	5/20	8/20	14/20§
LBA1334 pAGS <i>cpsl</i>	0/19	2/19	6/19	11/19§

Trifolium repens L. (white clover) seedlings were grown with their roots on filter paper in Petri dishes containing Jensen-1% agar¹⁴ supplemented with 0.5 mM Ca(NO₃)₂. Transformation was achieved by superficially wounding the stems of 6–8-day-old seedlings 2 mm under the first pair of leaves with the tips of an electron microscope grid forceps (number 7, Dumont, Switzerland) carrying *A. rhizogenes*. Bacteria were grown on LC (ref. 24) plates containing full antibiotic concentrations (see Fig. 1 legend) for Ar1334, Ar1334 carrying pBin19 or Ar1334 harbouring pAGS *cpsl* or pBin19 *psl*. Following transformation, the main root was excised 2–3 mm under the wound and the first hairy roots emerged from the wound sites 4–5 days later in all plants. Seedlings were transferred to fresh nutrient plates 5–7 days after wounding and nodulation assays were performed as described previously¹⁴. For re-isolation of rhizobia, red nodules were surface-disinfected in 5% H₂O₂ for 10 min, rinsed in sterilized water and crushed in 250 µl B-medium²⁵ and plated on B-medium²⁵. Re-isolates from R/248 Rifr-induced nodules were rifampicin-resistant, in contrast to re-isolates from R/843-induced nodules. Colonies from re-isolated rhizobia were blotted to nitrocellulose sheets and allowed to react with monoclonal antibody (mAb) 3, specific for the O-antigen of R/248 (ref. 21). Only re-isolates from R/248 Rifr-induced nodules were recognized by mAb 3. Rhizobial cell envelopes were analysed by SDS-polyacrylamide gel electrophoresis as previously described²⁶. A protein of relative molecular mass 45,000 and characteristic of R/248 Rifr (control) was present in bacteria re-isolated from R/248 Rifr-induced nodules and absent from re-isolates from R/843-induced nodules. Nodulation of *Vicia sativa* L. by R/248 Rifr re-isolates and their failure to nodulate white clover roots confirmed that *R. leguminosarum* bv. *viciae* 248 Rifr nodulates hairy roots of white clover co-transformed with pBin19 *psl* or pAGS *cpsl*. White clover seeds of cultivar 'Dutch Clover' (6990.6300) were purchased from Kieft, Blokker, The Netherlands.

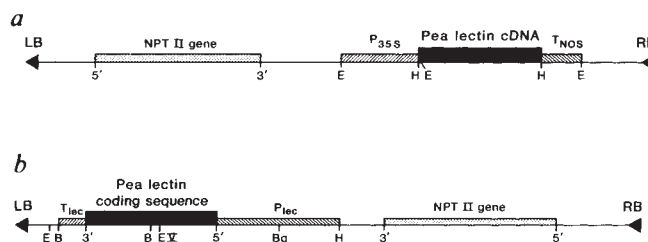
* Days after inoculation with rhizobia.

† Red nodules.

‡ Pseudonodules.

§ Red and white nodules and pseudonodules.

FIG. 1 Insertion of the pea lectin gene (*psl*) into binary vectors. To subclone the lectin cDNA as a *Hind*III fragment between the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) polyadenylation signal of the binary vector pAGS HB 35S (a derivative of pAGS127) (ref. 13), *psl* cDNA (from plasmid pMS2) (ref. 12) coding for a segment of the lectin's leading signal and the complete β - and α -chains, was first cloned into pIC-19H (ref. 7). The binary vector carrying the chimaeric pea lectin gene is pAGS *cpsl* (Fig. 1a). The complete genomic gene for *psl* (*lecA*) (ref. 7) was isolated as a *Hind*III-*Eco*RI restriction fragment and cloned into pBin19 (ref. 11). The resulting vector is termed pBin19 *psl* (Fig. 1b). Binary vectors were mobilized from *Escherichia coli* to *A. rhizogenes* LBA1334 (Ar1334) in a triparental mating using pRK2013 (kept in HB101 cells) as a helper plasmid²⁴. Ar1334 is a derivative of *A. tumefaciens* strain C58C9 with a chromosomal marker for rifampicin resistance and harbours the agropine type pRi1855 plasmid equipped with a spectinomycin resistance marker⁵. Ar1334 cells harbouring pAGS *cpsl* and Ar1334 cells carrying pBin19 *psl* were kept on LC plates²⁴ containing rifampicin (20 μ g ml⁻¹) plus tetracycline (2 μ g ml⁻¹), and rifampicin (20 μ g ml⁻¹) plus kanamycin (100 μ g ml⁻¹), respectively. To check if the binary vectors were present in Ar1334, plasmid DNA was isolated²⁸ and immediately transformed to MH1-competent cells. Plasmid preparations from *E. coli* and analysis of restriction patterns showed that both binary vectors were maintained in Ar1334 cells. Restriction enzyme digestions, agarose gel electrophoresis, dephosphorylation, annealing of restriction fragments and transformation to *E. coli* followed procedures described elsewhere²⁹. Isolation of restriction fragments was performed with glass beads³⁰ (GeneClean, Bio 101). Restriction sites are denoted: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; EV, *Eco*RV; H, *Hind*III. Other abbreviations: LB, left border; RB, right border; NPTII gene, neomycin-phosphotransferase II gene; P35S, CaMV 35S promoter; Tnos, nopaline synthase terminator.



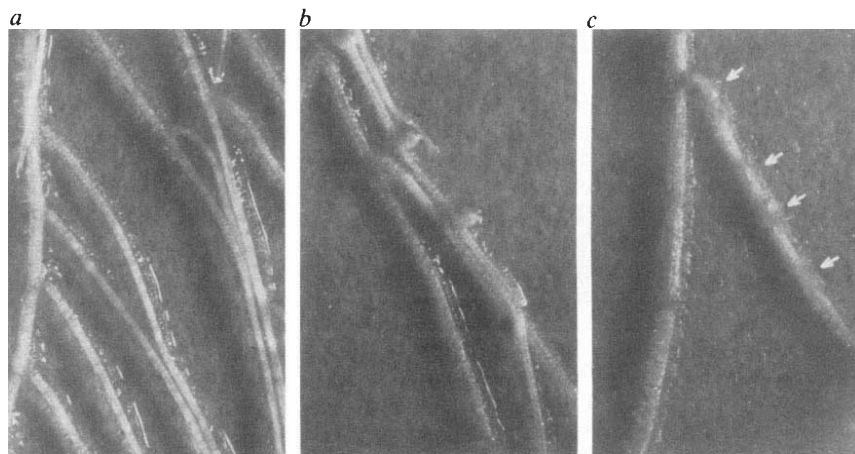
were induced on a set of clover seedlings which were grown on filter paper for 13 weeks in large Petri dishes (15 cm in diameter), filled with 100 ml plant growth medium¹⁴ containing 5 mM KNO₃ and 1% agar (see Table 1). The dot assay for NPTII activity¹⁵ was performed with extracts from randomly chosen hairy roots weighing from 50–100 mg. Hairy roots induced by Ar1334 (or adventitious roots) did not have any NPTII activity, whereas 86% (of a total of 64 samples) of hairy roots induced by Ar1334 harbouring pBin19 *psl* did have NPTII activity. Similar percentages of co-transformation (70–80%) using pBin19 contained in *A. rhizogenes* LBA9402 have been reported for several plant species¹⁶. The enzymatic assay was also positive for 42% (of a total of 66 samples) of the hairy roots induced by Ar1334 carrying pAGS *cpsl*. These results show that the binary-vector system of transformation is an efficient tool for introducing foreign genes into host plants.

To study the nodulation properties of white clover hairy roots, young hairy roots, 1–3 cm in length, were inoculated with *R. leguminosarum* bv. *trifolii* ANU843 (Rt843) (ref. 17). As with normal young white clover seedlings, nodules emerged 4–6 days

after inoculation on the roots of clover transformed with Ar1334, Ar1334 carrying pBin19 and Ar1334 harbouring pBin19 *psl* or pAGS *cpsl*. Nodules continued to be initiated along the first formed hairy roots and even on hairy roots which emerged 4–6 weeks after transformation. With few exceptions, nodules were red and the acetylene reduction assay¹⁴ indicated that nodules on hairy roots could reduce atmospheric nitrogen. Nodules were distributed on all hairy roots, and plants had 12–100 nodules. Most of the nodules remained small, particularly in heavily nodulated hairy roots. Electron micrographs of these nodules¹⁸ showed no differences with nodules induced by Rt843 on normal white clover roots with respect to the morphology, and the number and shape of bacteroids. These results show that white clover hairy roots behave as normal roots in nodulation, and that nodulation of white clover hairy roots by the homologous symbiont is not in any way inhibited by the presence of the binary vector T-DNA with or without the *psl* gene.

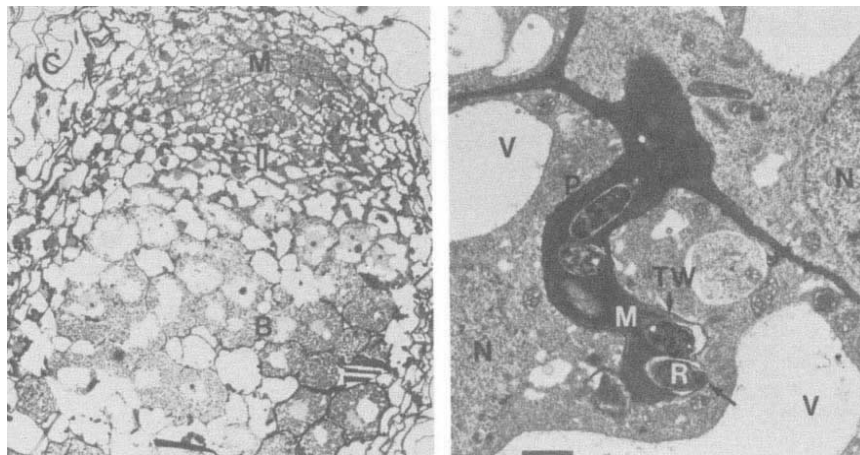
Rifampicin-resistant *R. leguminosarum* bv. *viciae* strain 248 (Rl248 RifR) (ref. 19) did not nodulate white clover hairy roots induced by Ar1334 or by Ar1334 carrying pBin19 without the

FIG. 2 Nodulation of white clover hairy roots co-transformed with the pea lectin gene by *Rhizobium leguminosarum* bv. *viciae*. Hairy roots induced by Ar1334 or by Ar1334 carrying pBin19 were not nodulated by Rl248 RifR (Fig. 2a). The host range of nodulation of white clover hairy roots, co-transformed with the genomic or copy DNA of *psl*, however, is extended to include *R. leguminosarum* bv. *viciae* 248 RifR. Of the nodulated plants co-transformed with pBin19 *psl* or with pAGS *cpsl*, 30% had 1–4 red nodules, whereas 5–10% had 5–12 red nodules. These nodules could not be distinguished from nodules induced by Rt843 on Ar1334-induced hairy roots. Some of the nodules formed after inoculation with Rl248 RifR were red as soon as they emerged and matured within 4–8 days (b), others emerged white, grew out in 8–16 days and then turned red. The assay for acetylene reduction¹⁴ was positive, indicating that red nodules induced by Rl248 RifR were able to reduce atmospheric nitrogen. All nodulated plants (Table 1) had ~1–50 white, small outgrowths, such as those indicated by arrows in c. In some cases these outgrowths developed



into white or red nodules; in most cases they did not grow further and were categorized as pseudonodules.

FIG. 3 Electron microscopy of red nodules induced by *Rhizobium leguminosarum* bv. *viciae* on white clover hairy roots co-transformed with the pea lectin gene. Adjacent red nodules on co-transformed roots were chosen for rhizobia re-isolation and identification, and processing for electron microscopy^{18,23}. Examination of the micrographs showed that the ultrastructure of nodules induced by *R*/248 Rif^R did not differ from that of nodules induced by *R*t843. *a*, Longitudinal section of a young nodule formed on a hairy root of a clover plant co-transformed with the complete pea lectin genomic gene. The meristematic (M), infection (I) and bacteroid (B) zones are well distinguished; C is the nodule cortex. Scale bar, 1 μ m. *b*, A longitudinal section of an infection thread as observed in the infection zone. Note the continuity of the host's plasmalemma (P) delimitating the thread matrix (M) and the gradual decrease of the thread wall material (TW) towards the tip of the infection thread. Rhizobia (R) are seen being released into the host cell cytoplasm by endocytosis (arrow). V, vacuole; N, nucleus. Scale bar, 100 μ m.



psl gene (Fig. 2a, Table 1). Moreover, infection threads could not be observed by microscopical light-examination of methylene blue-stained hairy roots. In both cases, however, white clover hairy roots responded to inoculation with *R*/248 Rif^R with marked root-hair curling, as has been previously reported for normal clover roots²⁰. Forty to sixty days after inoculation the hairy roots of 1 in 20 plants presented one or two white outgrowths. Their examination by electron microscopy, however, failed to reveal the presence of infection threads and they were therefore categorized as pseudonodules. These results show that as with normal roots, nodulation of white clover hairy roots is host-specific. Moreover, the insertion of a functional NPTII gene into co-transformed white clover hairy roots did not affect this specificity.

The *psl* gene appeared to affect the host-range of nodulation. Hairy roots induced with *Ar*1334 carrying binary vectors either with the complete genomic *psl* or cDNA construction were inoculated with *R*/248 Rif^R. Nodules confined to one or two hairy roots and their lateral roots (Fig. 2b) began to emerge 20 days after inoculation (Table 1). Such a distribution of nodules is accounted for by (1) not all the roots of a transformed seedling being hairy roots, and (2) not all hairy roots containing the pea lectin gene. These two types correspond to the roots of non-transformed clover plants and to hairy roots induced by *Ar*1334 or by *Ar*1334 containing pBin19, respectively, which are both resistant to infection by *R*/248 Rif^R. Electron micrographs of red nodules (Fig. 3) showed that their structures were similar to those of nodules induced by *R*/248 Rif^R on *Vicia sativa* roots and by *R*t843 on *Trifolium repens* roots. The presence of stunted nodules, or pseudonodules (Fig. 2c) however, indicated that in many cases infection was aborted. Electron micrographs of those pseudonodules revealed degenerated nodule meristems, bacteria in intercellular spaces deep in the root cortex and aborted infection threads.

Rhizobia were re-isolated from red nodules on hairy roots that had been co-transformed with the *psl* gene (Table 1). The heterologous symbiont *R*/248 Rif^R was identified using five criteria: (1) the rate of growth on B-agar plates, (2) antibiotic resistance; (3) positive reaction with a monoclonal antibody specific for the O-antigen of *R*/248 (ref. 21); (4) SDS-PAGE pattern of cell envelope proteins; and (5) nodulation of *V. sativa* L. and failure to nodulate *T. repens* L⁵. From this identification we concluded that *R*/248 Rif^R is able to induce root nodules on white clover hairy roots co-transformed with the *psl* gene. Nodulation, however, is delayed and most nodules are abnormal.

Our results show that the host range in *Rhizobium*-legume symbiosis is at least partially determined by symbiont-root-

lectin interactions. The introduction of the gene encoding the glucose-/mannose-binding pea lectin in clover roots allows infection by *R. leguminosarum* bv. *viciae* to proceed beyond root-hair curling. This indicates that root lectin contributes to a mechanism by which the plant selectively allows a homologous *Rhizobium* to penetrate root cells and induce infection threads. Our results also provide the first example of a host-specificity barrier in a plant-bacterium interaction being broken by genetic engineering of the host plant. Interaction of host lectin with the homologous symbiont might either induce increased infectivity of the rhizobia²² or enable collective endocytosis of rhizobia in the root hair curl²³. It is also conceivable that the introduction of *psl* to clover induces a response to *R. leguminosarum* bv. *viciae* bacteria which does not directly involve pea lectin. The fact that lectins with a sugar specificity similar to pea lectin are specifically present in plants of the pea cross-inoculation group, however, suggests that this is unlikely. □

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Importance of a novel GABA_A receptor subunit for benzodiazepine pharmacology

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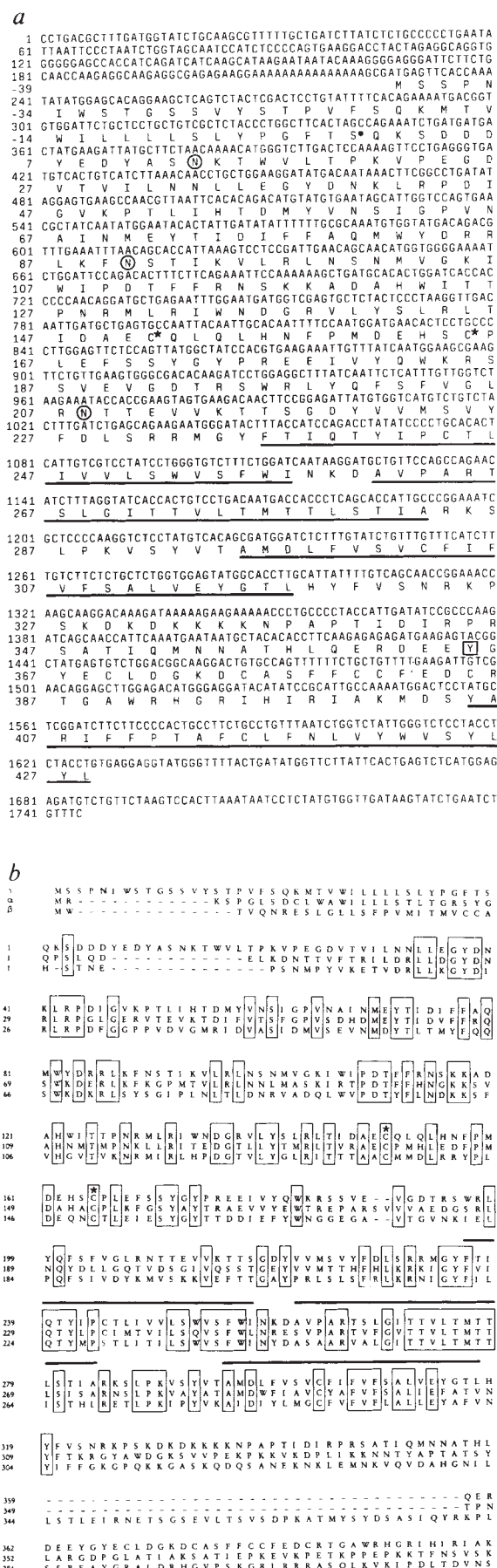
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NEUROTRANSMISSION effected by GABA (γ -aminobutyric acid) is predominantly mediated by a gated chloride channel intrinsic to the GABA_A receptor. This heterooligomeric receptor¹ exists in most inhibitory synapses in the vertebrate central nervous system (CNS) and can be regulated by clinically important compounds such as benzodiazepines and barbiturates². The primary structures of GABA_A receptor α - and β -subunits have been deduced from cloned complementary DNAs^{3,4}. Co-expression of these subunits in heterologous systems generates receptors which display much of the pharmacology of their neural counterparts, including potentiation by barbiturates³⁻⁵. Conspicuously, however, they lack binding sites for, and consistent electrophysiological responses to, benzodiazepines^{4,5}. We now report the isolation of a cloned cDNA encoding a new GABA_A receptor subunit, termed γ 2, which shares approximately 40% sequence identity with α - and β -subunits and whose messenger RNA is prominently localized in neuronal subpopulations throughout the CNS. Importantly, coexpression of the γ 2 subunit with α 1 and β 1 subunits produces GABA_A receptors displaying high-affinity binding for central benzodiazepine receptor ligands.

Cloned cDNA encoding a new GABA_A receptor subunit (Fig. 1a), designated γ 2, was isolated from a human fetal brain cDNA library by screening with a probe based on the conserved octameric amino-acid sequence in the second transmembrane region of GABA_A and glycine receptor subunits^{3,4,6}. The predicted primary structure of 468 amino-acid residues shares many features with α - and β -subunits (Fig. 1b). These include a signal peptide, a disulphide-bonded β -structural loop and three potential *N*-linked glycosylation sites in the putative extracellular

FIG. 1 The human GABA_A receptor γ 2 subunit. *a*, Nucleotide and predicted amino-acid sequence of cloned γ 2 cDNA. The amino-acid sequence of the encoded polypeptide is shown in the single letter code below the nucleotide sequence. Numbers on the left denote nucleotides and amino-acid residues. Negative numbers specify residues in the proposed signal sequence²⁴. Putative *N*-linked glycosylation sites are circled, the two cysteines involved in the formation of the invariant loop structure are starred, the four transmembrane segments are underlined and the putative tyrosine phosphorylation site is boxed. *b*, Comparison of the amino-acid sequences of human GABA_A receptor α 1, β 1 and γ 2 subunits. The amino acids of the mature polypeptides are numbered, identical residues are boxed. Transmembrane segments are overlined. Gaps are introduced for optimal sequence alignment. METHODS. A λ gt10 human fetal brain cDNA library¹¹ was screened for GABA_A receptor subunits using as a probe a 96-fold degenerate synthetic 23-mer oligonucleotide encoding a conserved octameric amino-acid sequence in the second transmembrane segment of GABA_A and glycine receptor subunits^{3,4,6}. A duplicate of the library was screened using a mixture of oligonucleotides which contain unique sequences found in previously cloned GABA_A-receptor subunit-encoding cDNAs (refs 3, 4 and unpublished observations). Cloned cDNAs hybridizing with only the 23-mer probe were sequenced^{25,26}. The γ 2 cDNA represents one of several new sequences identified in this manner.



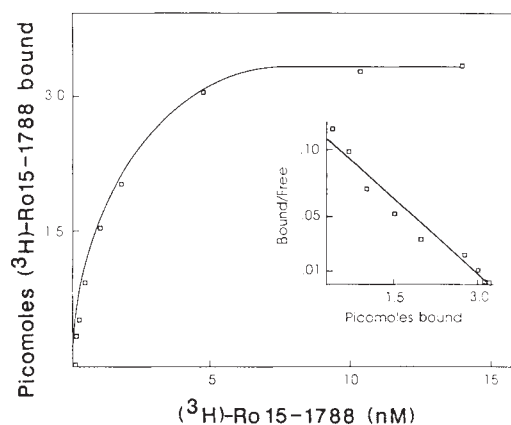


FIG. 2 Saturation isotherm of [^3H]Ro15-1788 binding. Inset, the corresponding Scatchard plot ($K_d = 0.97 \text{ nM}$, $B_{\text{max}} = 3.2 \text{ pmol mg}^{-1} \text{ protein}$). In three independent transfection experiments K_d values of $1 \pm 0.2 \text{ nM}$ and B_{max} values of $2.7 \pm 0.7 \text{ pmol mg}^{-1} \text{ protein}$ were obtained.

METHODS. Human $\alpha 1$ -, $\beta 1$ - (ref. 11) and $\gamma 2$ -encoding cDNAs, subcloned individually into the expression vector pCIS2 (ref. 27), were used to transform human embryonic kidney 293 cells as described previously^{5,28}. After transfection (48 h), cells from ten plates (10 cm; 4×10^6 cells per plate) were washed twice with phosphate-buffered saline (PBS) and scraped into PBS (10 ml). The cell pellet (500g) was homogenized in a Polytron tissue homogenizer (Brinkmann) in 10 ml of 10 mM potassium phosphate, pH 7.4, and centrifuged (50,000g, 20 min). This procedure was repeated three times and the final pellet resuspended in potassium phosphate buffer, pH 7.4, containing 100 mM KCl. For each ligand concentration, triplicate homogenates, each equivalent to 10^6 cells (100 μg protein), were incubated (4 $^{\circ}\text{C}$, 60 min) in a 1 ml reaction volume with [^3H]Ro15-1788 (NEN, 75 Ci mmol $^{-1}$). Non-specific binding was determined in the presence of 10^{-6} M clonazepam and was $<10\%$ of total binding. K_i values of clonazepam, diazepam, and DMCM (see text) were determined in similar reactions using 10^{-10} – 10^{-5} M unlabelled competitor compounds. Samples were vacuum-filtered on GF/C filters with a vacuum filter apparatus (Biorad) and filters were washed twice with 5 ml homogenization buffer. After drying, filter-retained radioactivity was determined by liquid scintillation counting.

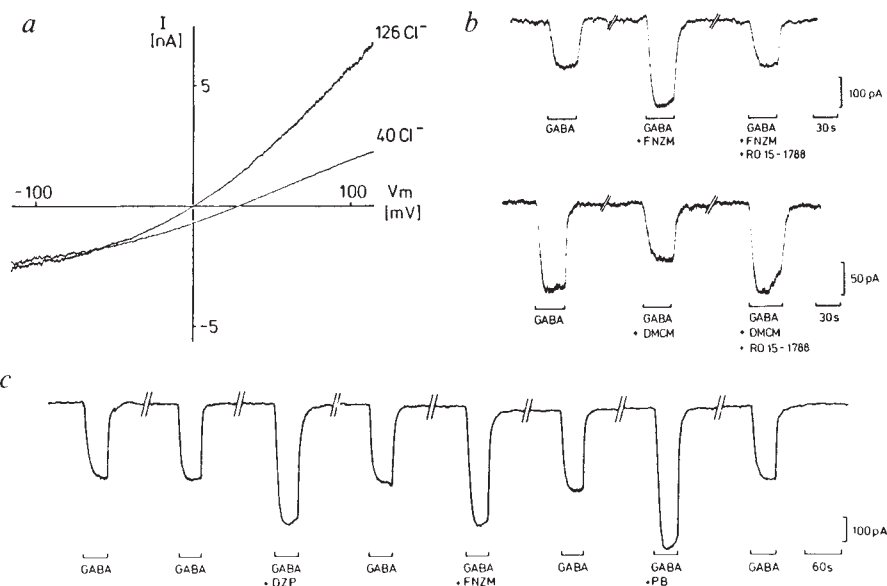
domain, as well as four transmembrane segments. The greatest sequence similarity to α - and β -subunits occurs in the region of these transmembrane segments and includes the charged amino-acid residues thought to form the channel mouth^{3,7}. The proposed large intracellular domain displays the highest sequence divergence among the subunits. In the $\gamma 2$ subunit, this domain contains a consensus sequence for tyrosine-specific protein phosphorylation, suggesting a target for the cellular control of channel activity by receptor-mediated tyrosine kinases⁸. A putative control site for serine phosphorylation by protein kinase A is found within the same domain of the $\beta 1$ subunit³. The overall sequence similarity to $\alpha 1$ and $\beta 1$ subunits is 42 and 35%, respectively. This level of structural similarity is seen for the different nicotinic acetylcholine receptor subunits⁹ and is significantly below the 70–80% identity which typically characterizes the variants of GABA $_A$ receptor α -subunits^{3,4} or β -subunits (manuscript in preparation). Based on these structural considerations and the functional signature detailed below, the

$\gamma 2$ polypeptide is a novel GABA/benzodiazepine receptor subunit.

To determine the functional properties of the $\gamma 2$ subunit, the cloned cDNA was transiently expressed in cultured human cells⁵, either singly or together with cloned cDNA encoding human $\alpha 1$ and $\beta 1$ subunits¹¹. The membranes of the transfected cells were analysed for the binding of radiolabelled ligands specific for central benzodiazepine receptors^{2,10}. High-affinity binding sites for [^3H]Ro15-1788 ($K_d = 1.2 \pm 0.2 \text{ nM}$, $B_{\text{max}} = 2,900 \pm 300 \text{ fmol mg}^{-1}$; Fig. 2) and [^3H]flunitrazepam ($K_d \sim 2 \text{ nM}$, $B_{\text{max}} \sim 2,000 \text{ fmol mg}^{-1}$; data not shown) were observed on cells expressing all three GABA $_A$ receptor subunits. The binding of these labelled compounds was completely blocked in the presence of $1 \mu\text{M}$ diazepam ($K_i = 10 \pm 2 \text{ nM}$), clonazepam ($K_i = 1.1 \pm 0.7 \text{ nM}$) or methyl-4-ethyl-6,7-dimethoxy- β -carboline-3-carboxylate (DMCM; $K_i = 2.1 \pm 0.8 \text{ nM}$; data not shown). The K_i values of these compounds are similar to their rank order and potency determined on cerebellar membranes¹². No

FIG. 3. Ionic and pharmacological characteristics of GABA-induced membrane currents in 293 cells expressing human GABA $_A$ receptor $\alpha 1$, $\beta 1$ and $\gamma 2$ subunits. *a*, Current-voltage relation of GABA-induced responses (GABA, $100 \mu\text{M}$). A reversal potential close to 0 mV was seen in 126 mM $[\text{Cl}^-]_o$. When $[\text{Cl}^-]_o$ was lowered to 40 mM the reversal potential shifted to 30 mV, indicating a highly chloride-selective conductance. *b*, Flunitrazepam (FNZM, $1 \mu\text{M}$) reversibly potentiated the GABA-induced current (GABA, $5 \mu\text{M}$). This potentiation was completely inhibited by Ro15-1788 ($1 \mu\text{M}$). DMCM ($1 \mu\text{M}$) reduced the GABA-induced current by $\sim 50\%$. This reduction was lost in the presence of Ro15-1788 ($1 \mu\text{M}$). *c*, Diazepam (DZP, $1 \mu\text{M}$), FNZM ($1 \mu\text{M}$) and pentobarbital (PB, $50 \mu\text{M}$) all reversibly potentiated the current induced by $10 \mu\text{M}$ GABA (// indicates a 5-min interval between subsequent applications).

METHODS. Membrane currents were recorded by the patch-clamp technique in the whole cell configuration¹³, using an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt). Cells were clamped at -60 mV . Data were filtered at 3 KHz (8-pole Bessel) and sampled up to 10 KHz. To determine the reversal potential of the transmitter-induced responses, membrane currents were measured while applying a voltage ramp (-120 to 120 mV , 1.6 s duration). The current-voltage curves were calculated by subtracting currents (I) before application of GABA from currents obtained in the presence of GABA, and were plotted as a function of membrane potential (V_m). Substances were added, as indicated by bars, at the following concentrations: FNZM, DZP, Ro15-1788, and DMCM,



$1 \mu\text{M}$; PB, $50 \mu\text{M}$. Pipettes contained in mM: CsCl, 130; MgCl_2 , 1; CaCl_2 , 0.5; EGTA, 5; HEPES, 10; pH, 7.2. Cultures were continuously perfused with a solution containing in mM: KCl, 5.4; NaCl, 116; MgCl_2 , 0.8; CaCl_2 , 1.8; D-glucose, 11.1; NaHCO_3 , 26.2; HEPES, 5; pH, 7.2. All measurements were performed at room temperature.

binding was detected when the $\gamma 2$ subunit alone or pairs of subunits were expressed. These data show that α -, β - and γ -subunits can assemble into GABA_A receptors which contain a high-affinity benzodiazepine binding site.

The receptors formed by co-expression of three different subunits were characterized by electrophysiology, using the whole cell patch-clamp technique¹³ (Fig. 3). The application of GABA induced large inward currents; GABA sensitivity showed a Hill coefficient of ~ 1 , as observed upon co-expression of α - and β -subunits⁴. The selectivity for chloride ions is demonstrated by a shift in the current versus voltage curve when changing the concentration of extracellular chloride. The GABA-induced current was blocked by the GABA_A receptor antagonist bicuculline (10^{-5} M) and the channel blocker picrotoxin (10^{-4} M) (data not shown) but was potentiated by the barbiturate pentobarbital. Importantly, these receptors display the full functional properties of GABA/benzodiazepine receptors. In all expressing cells tested, responses to GABA were consistently enhanced about

twofold by the benzodiazepine receptor agonists flunitrazepam and diazepam and reduced by 50% in the presence of the inverse agonist DMCM^{2,14}. These effects were completely blocked by the benzodiazepine receptor antagonist Ro15-1788. This spectrum of sensitivity was not observed upon co-expressing $\alpha 1\gamma 2$ and $\beta 1\gamma 2$ subunit pairs. Furthermore, no responsiveness was seen on expression of the $\gamma 2$ subunit alone which generated homomeric receptors (data not shown) comparable in their properties to the receptors formed from single α - or β -subunits⁵. Our results indicate that all three subunits contribute to the formation of GABA/benzodiazepine receptors.

The $\gamma 2$ subunit is comparable in abundance to α - and β -subunits, as judged from the number of cognate cDNA clones in libraries of rat, bovine and human CNS origin and as evidenced by northern analysis of rat and bovine brain mRNA (unpublished observations). To determine the spatial pattern of $\gamma 2$ mRNA expression in the CNS, we performed *in situ* hybridization on sagittal sections of rat brain, using as a probe a radioac-

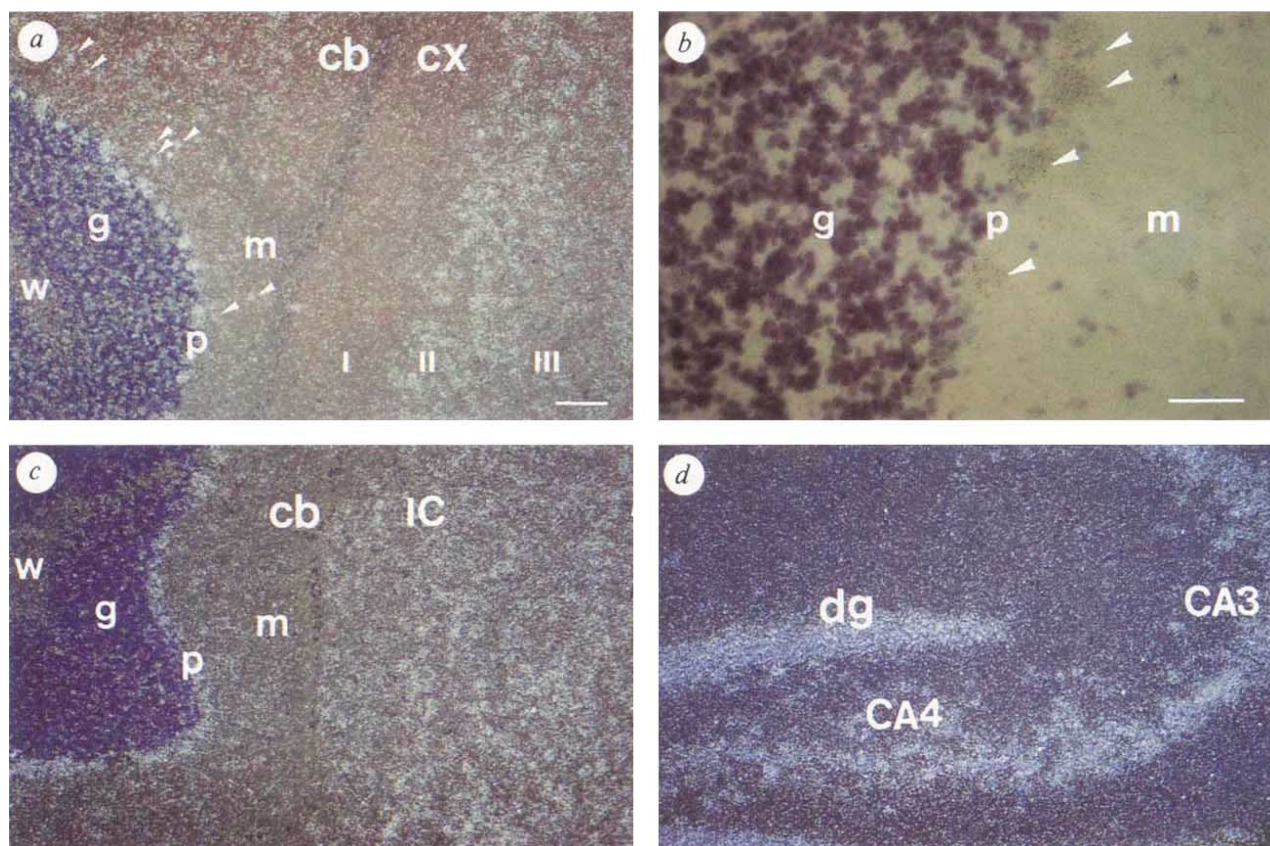


FIG. 4 Location of neurons synthesizing $\gamma 2$ subunit mRNA in rat brain. *In situ* hybridization was performed using a ^{35}S -labelled cRNA on frozen, paraformaldehyde-fixed brain sections. Grain clusters in the emulsion mark the location of cells containing hybridizing mRNA in stained sections. Sections probed with ^{35}S -labelled sense RNA produced no hybridization pattern (data not shown). *a*, Darkfield photomicrograph of cerebellar cortex (cb, left) and neocortex (cx, right). In cerebellar cortex, $\gamma 2$ subunit mRNA expression is highest in the Purkinje neurons in the Purkinje cell layer (p) with an additional population of hybridizing cells (arrowheads) in the molecular layer (m) representing presumptive basket/stellate neurons. In the neocortex, numerous cells throughout layers II–VI express the $\gamma 2$ subunit mRNA highly and display no obvious lamination pattern; neocortical layers I–III are shown. *b*, Bright-field photomicrograph of $\gamma 2$ subunit mRNA expression in all Purkinje neurons (arrowheads). *c*, Dark-field photomicrograph of cerebellar cortex (left) and of the inferior colliculus (IC, right). Numerous cells expressing the $\gamma 2$ -subunit mRNA are distributed throughout the inferior colliculus, unlike the superior colliculus (not shown). *d*, Dark-field photomicrograph of part of the hippocampal formation showing high $\gamma 2$ subunit mRNA expression in granule neurons of the dentate gyrus (dg) and pyramidal neurons of the hippocampal CA3 and CA4 regions. CA1 and CA2 pyramidal neurons (not shown) similarly express $\gamma 2$ -subunit mRNA. Scale bar in *a* (for *a*, *c* and *d*),

100 μm ; in *b*, 30 μm . Other abbreviations: g, granule cell layer; w, white matter.

METHODS. RNA probes were transcribed from a linearized Bluescript plasmid DNA, containing a 1,500-base pair *EcoRI* fragment from the coding region of rat $\gamma 2$ cDNA. Following plasmid linearization at unique restriction sites in the polylinker region, sense or antisense RNA was synthesized using phage T3 or T7 RNA polymerase. RNA products were radiolabelled²⁹ to a specific activity of $\sim 3 \times 10^8$ c.p.m. μg^{-1} using 1.0 μg linearized plasmid DNA, 50 μCi α - ^{35}S CTP (1,000 Ci mmol^{-1} , Amersham), 2.5 mM each of ATP, GTP and UTP, and were hydrolysed (pH 10, 37 $^{\circ}\text{C}$, 1 h) to an average length of 100 nucleotides. For each section the RNA probe (2.5×10^5 c.p.m.) was dissolved in 50 μl hybridization solution which included 0.6 M NaCl, 50% formamide, and 40 mM β -mercaptoethanol. *In situ* hybridization was performed as described previously³⁰. Briefly, the sections were hybridized at 42 $^{\circ}\text{C}$ for 3 days, washed twice for 20 min each at 65 $^{\circ}\text{C}$ in $0.1 \times \text{SSC}$, 0.05% inorganic pyrophosphate, 14 mM β -mercaptoethanol, and dehydrated in alcohol. Following overnight exposure to Kodak XAR 5 film, the sections were dipped in Kodak NTB2 emulsion (diluted 1:1 in water), exposed for 8 days, and stained in 1% Fast Green and 0.5% cresyl violet. Sagittal brain structures were identified from ref. 31.

tively labelled cRNA derived from cloned rat $\gamma 2$ cDNA (manuscript in preparation). Our results show that $\gamma 2$ mRNA is prominently expressed in neuronal subsets throughout the CNS which include neurons in the olfactory bulb, anterior olfactory nuclei, preoptic area, neocortex, globus pallidus, hippocampus, dentate gyrus, thalamus, inferior colliculus, substantia nigra, pontine nuclei, cerebellar cortex and cerebellar nuclei. Four of these regions have been chosen to illustrate the cellular location of $\gamma 2$ mRNA (Fig. 4). Significantly, all of these regions contain high-affinity binding sites for benzodiazepines^{15,16} and also express α - and β -subunit mRNAs (refs 17, 18; B.D.S., unpublished observations), further supporting the hypothesis that the $\gamma 2$ subunit is an integral part of GABA/benzodiazepine receptors.

The existence of the $\gamma 2$ subunit was not anticipated from biochemical studies as subunits of affinity-purified GABA/benzodiazepine receptor are electrophoretically resolved into only two main bands corresponding to relative molecular mass (M_r) 48,000–53,000 (48K–53K) (α) and M_r 55–57K (β) (ref. 1). These bands, however, are heterogeneous, consisting of variants of the α -subunits^{3,4} and β -subunits, and of additional GABA_A receptor subunits, including $\gamma 2$ and a related $\gamma 1$ subunit, whose cloning preceded that of $\gamma 2$ and which shows glial localization (manuscript in preparation). The mature $\gamma 2$ -polypeptide (unglycosylated, $M_r \approx 48$ K) may co-migrate with α -subunits (M_r , 48–53K)^{1,4}, which have been postulated to carry the benzodiazepine site on the basis of photoaffinity labelling with flunitrazepam^{21–23}. Our results on benzodiazepine responsiveness indicate that the γ -subunit contributes to the formation of the benzodiazepine site and thus may also be photoaffinity-labelled. In fact, it is probable that a flunitrazepam-labelled $\gamma 2$ subunit would be indistinguishable from certain labelled α -subunits by present methods.

We note that other subunit combinations may also create benzodiazepine responsiveness. Indeed, recent cDNA cloning experiments in our laboratory provide evidence for the existence of additional GABA_A receptor subunits (manuscript in preparation). It seems likely therefore that the true diversity of GABA/benzodiazepine receptor subtypes has been only partly revealed by classical pharmacology^{12,19,20}. □

Vaccination against ovine cysticercosis using a defined recombinant antigen

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CYSTICERCOSIS caused by larval tapeworms is a major public health problem and a cause of substantial economic losses in the farm-animal industries. *Taenia ovis* in sheep is a particularly important example. Immunity to reinfection with the larvae has a central role in regulating natural transmission of the parasites¹, and vaccination with antigens from the early larval oncosphere stage can induce complete protection against infection². As it is impractical to obtain enough oncospheres for a commercial vaccine against these tapeworms, an alternative approach is to use recombinant DNA methods to generate a cheap and plentiful supply of antigens. We report here the expression in *Escherichia coli* of complementary DNA encoding *T. ovis* antigens as fusion proteins with the *Schistosoma japonicum* glutathione S-transferase. Vaccination of sheep with these fusion proteins gave significant, although not complete, immunity against challenge infection with *T. ovis* eggs. Commercial development of a vaccine is being pursued.

Oncospheres of *T. ovis* were chosen as the source of messenger RNA for constructing a cDNA library because they are known to be a rich source of host-protective antigens^{3,4}. Furthermore, *in vitro* culture techniques⁵ have shown that hatched and activated *T. ovis* oncospheres secrete potent host-protective antigens^{6,7}, suggesting synthesis is active. Oncospheres were therefore treated with artificial gastric and intestinal fluid and incubated before extraction of mRNA. To reduce the number of antigen-expressing cDNA clones to be tested in vaccination trials in sheep, it was first necessary to identify probable candidates for host-protective antigens. Preliminary experiments with sera from immune sheep showed strong antibody recognition of oncosphere antigens of relative molecular mass (M_r) 47,000–52,000 (47–52K) (unpublished observations). The host-protective nature of the 47–52K antigens was demonstrated directly when lambs immunized with an SDS-PAGE cut-out of this region were protected significantly (98%) against challenge infection (Fig. 1). To isolate the genes encoding these antigens, rabbit antibodies specific for the 47–52K region were eluted from western blots and used to screen the expression library⁸. Figure 1 shows that the antibody probe was specific for the 47–52K region, and enabled us to isolate two clone types, designated 45S and 45W. Antigen- β -galactosidase (β -gal) fusion proteins prepared from the clones (β -gal-45S and β -gal-45W) failed to stimulate host-protective immunity in sheep even though antibody was produced to the 47–52K antigens (Fig. 1). This experiment, however, demonstrated that the 47–52K region contains a series of serologically-related molecules, and that the antigens, or parts of them, had been cloned.

Plasmid vectors have been constructed recently which express antigens as fusion proteins with the enzyme glutathione S-transferase (GST) of *Schistosoma japonicum*, thereby enabling

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affinity-purification under non-denaturing conditions⁹. Fusion proteins GST-45S and GST-45W were prepared using a prototype of the vectors, pSj10ΔBam7Stop7, and tested either separately or together in an immunization trial in lambs (Table 1, trial A). The results showed that GST-45W, either alone or in combination with GST-45S, stimulated significant host-protective immunity compared with the GST controls, whereas GST-45S alone was ineffective. The active component was therefore GST-45W, and a further vaccination trial was carried out using different adjuvants and doses of GST-45W. Significant levels of immunity were induced at all dose levels in oil, Freund's complete adjuvant or saponin adjuvants, the best being 94% protection with 50 μg GST-45W in saponin (Table 1, trial B).

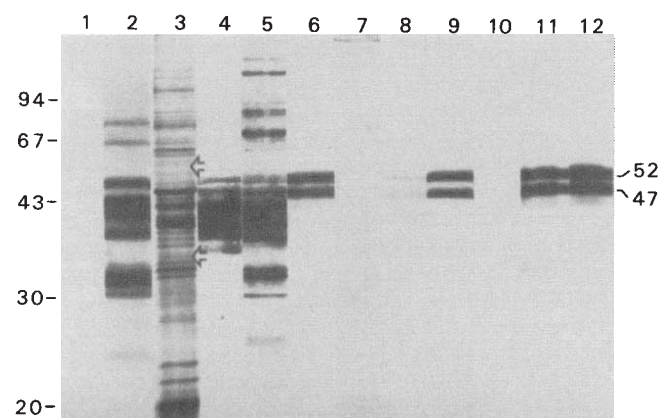


FIG. 1 Immunoblotting analysis of *T. ovis* oncosphere antigens. Lanes probed with: pooled sera from five sheep before immunization (1); pooled sera from five sheep after immunization with an extract of 200,000 oncospheres solubilized in 2% SDS and 65 mM dithiothreitol (2); pooled sera from three sheep immunized with gel cut-out fraction (4); serum from a rabbit hyperimmunized with oncosphere extract (5); affinity-purified antibodies eluted from blots containing antigens with relative mobilities of 47–52K (6); pooled sera from three sheep immunized with β-gal (7); pooled sera from three sheep immunized with β-gal-45S (8); pooled sera from three sheep immunized with β-gal-45W (9); pooled sera from four sheep immunized with GST (10); pooled sera from four sheep immunized with GST-45S (11); pooled sera from four sheep immunized with GST-45W (12). Lane 3 shows oncosphere extract stained with silver¹⁶ and the approximate size of gel cut-out injected into sheep is indicated between the arrows. Mean numbers of *T. ovis* cysticerci found in sheep injected with gel cut-out fraction, control polyacrylamide gel or whole oncosphere antigen in SDS plus gel were 1.0, 55.0 and 8.0, respectively. The sheep injected with the oncosphere gel cut-out were significantly ($P < 0.01$, 't' test) protected against infection.

METHODS. For vaccination, an extract from 7×10^6 oncospheres was separated by SDS-PAGE through a 5–25% polyacrylamide gradient¹⁷ and the fraction cut as shown. The fraction was homogenized with oil adjuvant¹⁵ and injected part subcutaneously and part intramuscularly into three sheep on two occasions, 2 weeks apart. Positive control sheep were injected with homogenized polyacrylamide gel containing whole oncosphere antigen (three sheep) and negative control sheep were immunized with homogenized polyacrylamide gel without antigens (four sheep). Two weeks after the second injection sheep were infected with 5,000 viable *T. ovis* eggs and 6 weeks later carcasses were examined for cysts. For analysis, oncosphere antigens were separated by SDS-PAGE through 12–18% polyacrylamide gradients. Electrophoretic transfer to nitrocellulose¹⁸ was followed by blocking for 2 h in 2% casein hydrolysate (Oxoid) 0.5% Tween 20 in 20 mM Tris-HCl buffer pH 7.4, containing 0.5 M NaCl (TBS). Sera were diluted 100-fold in 1% fish gelatin (Norland Products, USA) in TBS and incubated with blocked antigen strips overnight on a rocking platform. Strips were washed four times with TBS containing 0.05% Tween 20, and bound antibodies detected by incubation for 4 h with peroxidase-conjugated rabbit anti-sheep IgG (Cappel, Cooper Biomedical Inc.) or goat anti-rabbit IgG (Cappel) followed by washing and visualization with 4-chloro-1-naphthol (Biorad). Affinity-purified antibodies to the 47–52K antigens were prepared by elution of rabbit antibody from blots using 0.1 M glycine-HCl, pH 2.5 (ref. 8). Eluted antibodies were neutralized and fish gelatin carrier protein was added to 1% before concentration. The relative mobilities of standards are shown in the margins.

Although control sheep immunized with GST in saponin were not included in this trial, several experiments have since been carried out using saponin plus GST controls, with no non-specific protective effect being observed (unpublished observations).

Treatment of the fusion proteins with SDS and dithiothreitol substantially reduced protection (Table 1) indicating that the conformation of the fusion protein is important to its activity. Western blots of oncosphere antigen probed with sera from vaccinated sheep showed that antibody was directed predominantly against the 47–52K region (Fig. 1). The complete nucleotide sequence of the 928-base pair 45W cDNA is shown in Fig. 2. There is a single open reading frame in the sequence,

TABLE 1 Vaccination of sheep with glutathione S-transferase fusion proteins

Group	No. cysticerci in sheep	Mean	% Protection
Trial A			
Oncosphere antigen	0, 1, 1, 1	0.75	99
Buffer control	68, 95, 105, 109	94	
GST-45W + GST-45S	0, 15, 18, 59	23*	73
GST-45W + GST-45S	31, 36, 56, 141	66	22
SDS treated			
GST-45S	40, 74, 104, 161	95	—
GST-45W	11, 12, 24, 68	29*	66
GST	46, 69, 106, 116	84	
Trial B			
Oncosphere antigen	0, 0, 0, 0	0	100
GST oil	11, 16, 17, 18, 20		
	25, 29, 45, 48, 57	29	
GST-45W 50 μg	0, 0, 0, 0, 2		
oil	4, 6, 16, 18, 25	7†	75
GST-45W 300 μg	0, 0, 1, 7, 9	3*	88
oil			
GST-45W 50 μg	0, 0, 0, 1, 7	2†	94
saponin			
GST-45W 50 μg	0, 0, 0, 2, 29	6*	79
FCA			
GST FCA	14, 15, 28, 44, 46	29	

T. ovis eggs recovered from freshly purged tapeworms from dogs were washed thoroughly and stored at 4 °C for no more than 1 week. After hatching and activation^{4,5}, the oncospheres were maintained *in vitro* for 2–3 h in DMEM, washed twice in sterile physiological saline, frozen and stored in liquid nitrogen. Approximately 30×10^6 oncospheres were ground to a paste in liquid nitrogen and RNA isolated after solubilization in guanidine-HCl (ref. 10). Pooled total RNA (20–50 μg) was fractionated over oligo(dT) cellulose¹¹. Polyadenylated RNA (400 ng) was transcribed into double-stranded cDNA using AMV reverse transcriptase and ribonuclease RNase H (Amersham cDNA synthesis system RPN.1256), methylated with *EcoRI* methylase (New England Biolabs) and ligated to *EcoRI* linkers (5'-GGAATTC-3') (New England Biolabs). The cDNA was cloned¹² into λgt11 yielding a library of 7.5×10^5 individual recombinants. Fifty-thousand phage plaques were screened¹² with affinity-purified anti-47–52K antibodies (Fig. 1) and five clones were identified, three of which were strongly immunoreactive and demonstrated to be siblings by direct sequencing. This clone type was designated λgt11.45S. The other two clones were weakly immunoreactive and similarly grouped into a single class called λgt11.45W. The inserts from the 45S and 45W λgt11 clones were ligated into *EcoRI*-cleaved pSj10ΔBam7Stop7 (ref. 9) and transformed into *E. coli* strain JM109 (ref. 14). Fusion proteins were prepared from these constructs, after transformation, by affinity chromatography on glutathione agarose⁹ (Sigma) and stored at –70 °C before use in the vaccination trials. **Trial A.** The fusion proteins, oncosphere antigen, and GST control protein were emulsified in oil adjuvant¹⁵ and injected subcutaneously into groups of four sheep on three occasions, 3 weeks apart. Total protein in the three doses of fusion protein amounted to 50 μg per sheep based on A_{280} estimation⁹. Solubilized oncosphere antigen equivalent to 150,000 oncospheres was injected in oil adjuvant in each positive control sheep. Three weeks after the third injection, all sheep were infected with ~5,000 viable *T. ovis* eggs and 6 weeks after infection, sheep carcasses were examined for cysts. **Trial B.** In the second trial sheep were immunized with three injections of GST-45W in oil adjuvant or with 1 mg Saponin HP2 (PPF) injected subcutaneously; or with Freund's complete adjuvant (FCA, Difco) intramuscularly on first injection, and with the second and third injections subcutaneously in incomplete adjuvant. Control positive sheep were immunized with oncosphere antigen as in Trial A. Two weeks after the third injection all sheep were infected with ~2,000 viable *T. ovis* eggs and 6 weeks after infection sheep carcasses were examined for cysts. The challenge infection with *T. ovis* eggs was deliberately decreased compared with trial A to mimic more closely a field infection. Despite the difference in challenge dose a similar per cent protection was observed in the GST-45W (oil adjuvant) groups of trials A and B.

— No protection relative to GST controls.

* Significantly different from buffer and GST controls at $P < 0.05$ ('t' test).

† Significantly different from buffer and GST controls at $P < 0.01$ ('t' test).

EcoRI	
1	GAATTC CCG GAC TAC GAA CAA CCC ATC GAG AGA ACA GTG GTA GAA Pro Asp Tyr Glu Gln Pro Ile Glu Arg Thr Val Val Glu 13
46	TAT CCA TCA CTA CGT GAC ATC TTT GCT TGG GAA CCT CCG ACT TCT Tyr Pro Ser Leu Arg Asp Ile Phe Ala Trp Glu Pro Pro Thr Ser 28
91	AAC TCC ATT GGC CTA ACT TGG CAA AGG CAT GCA TTT CCT GGT GTG Asn Ser Ile Gly Leu Thr Trp Gln Arg His Ala Phe Pro Gly Val 43
136	GAA CGT GAA GTG CTC ACA TTG AAG GCA GTG CCG ACT TCT GAA CCC Glu Arg Glu Val Leu Thr Leu Lys Ala Val Pro Thr Ser Glu Pro 58
181	AAT AAC ACC AAG ACA GCA TAT GCA AAG CTC GGC AGC GGA AAA GTC Asn Asn Thr Lys Thr Ala Tyr Ala Lys Leu Gly Ser Gly Lys Val 73
226	ACT CTT GAT GGA CTG AAG CCC AAT GCC ACA TAT CTT GTG ACT GCG Thr Leu Asp Gly Leu Lys Pro Asn Ala Thr Tyr Leu Val Thr Ala 88
271	ACG GCA AAT ATA AGT GGA GAC ACA ATT CTG GTA TTG AGC AAT ACT Thr Ala Asn Ile Ser Gly Asp Thr Ile Leu Val Leu Ser Asn Thr 103
316	TTT CAT ACA CTG GCC AAT GGC ACA AAT ATT ATA AAT AAC ATC TTC Phe His Thr Leu Ala Asn Gly Thr Asn Ile Ile Asn Asn Ile Phe 118
361	CAT TGG GGT CCT GTG ACT AAT CAA TCA ATT CAA GTA AGA TGG GAT His Trp Gly Pro Val Thr Asn Gln Ser Ile Gln Val Arg Trp Asp 133
406	CAG ATA AAA CCG GAG GAA ACA AGC GCT CTG ATA GTC ACA CTG ACG Gln Ile Lys Pro Glu Glu Thr Ser Ala Leu Ile Val Thr Leu Thr 148
451	GCA GAG GTG GCT TCT GAC CCC GGA GTG GAA AGA TCG GAG TCT GCA Ala Glu Met Ala Ser Asp Pro Gly Val Glu Arg Ser Glu Ser Ala 163
496	CTC TTC GGT AAA GGA AAG GTC ACT GTT GAC GGA CTG GAG TCC GAC Leu Phe Gly Lys Gly Lys Val Thr Val Asp Gly Leu Glu Ser Asp 178
541	ACA CTA TAT ATT GCG ACT GTG ATG GTA TTT AGA AAT GGA AGG CAA Thr Leu Tyr Ile Ala Thr Val Met Val Phe Arg Asn Gly Arg Gln 193
586	TAC TTC AAT TCC ACC AGA GAT ATT CGA ACA CTC AAA TCT GGC CAT Tyr Phe Asn Ser Thr Arg Asp Ile Arg Thr Leu Lys Ser Gly His 208
631	AAG GAG GTA ACA GTC GTA ACA ACT AGT GGA TCT GGT ATA GCC TCC Lys Glu Val Thr Val Val Thr Thr Ser Gly Ser Gly Ile Ala Ser 223
676	ACA ATA CTT GGA CTC CTC ACC TGC GTG GCG CTA GTC CTT GCT Thr Ile Leu Gly Leu Leu Leu Thr Cys Val Ala Leu Val Leu Ala 238
721	TGAACACTTGGCTCGGTCAATGCCCATTTCCAAACCATCCATCTTCAATCTCAGC ---
779	TCCCATGACTTGCTTGTCTGACCAACCTCTTCTACCTTGACGCACTCATGGTGTCCG
839	GAGTGCCCTCTCCCTACTCATCTTGTCTCAACTAATATGGCTTGACACCTCTTGATG
898	GATAACCACTGAATGGCAAATAAACAATTC
EcoRI	

FIG. 2 Nucleotide sequence and predicted amino-acid sequence of the 45W *EcoRI* cDNA. The DNA sequence of 928 nucleotides is numbered on the left starting at the *EcoRI* linker sequence and the TGA termination codon and putative polyadenylation signal, AATAAA, are indicated. The amino-acid sequence is given below the DNA sequence and its numbering is on the right. METHODS. The *EcoRI* fragment of λ gt11-45W containing *T. ovis* cDNA was cloned in both orientations into phage M13mp18 (ref. 19) for sequencing. Overlapping deletions of the cDNA insert cloned into M13mp18 were obtained by exonuclease III digestion as described by Henikoff²⁰. Single-stranded DNA was prepared and both strands were sequenced by the dideoxy chain-termination method²¹.

maintaining the frame of both λ gt11 and pSj10 Δ Bam7Stop7. The predicted 25.83K peptide agrees with size estimates (26K) of the fusion protein on SDS-PAGE (data not shown), but differs substantially from the size of the native antigens, which may be post-translationally modified. The absence of a consensus translational start signal (ATG) indicated that the cDNA was not a complete copy of the 45W mRNA. Although the extent of the 5' end of the specific mRNA has not been determined, the potential upstream regions do not seem to be necessary for effective vaccination. Indeed, the protective epitope(s) may comprise only a small region of 45W; preliminary sequence data indicate that the non-protective, ~500-base pair 45S clone is a shorter version of 45W, so that further comparative analyses may allow definition of the host-protective epitope(s).

This report describes the first identification of a protective antigen of the taeniid cestodes produced by recombinant DNA methods. Although 100% protection was not achieved, the levels that were attained should enable a significant level of parasite control in the field and could form the basis of a commercial vaccine. □

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Identification of susceptibility loci for insulin-dependent diabetes mellitus by trans-racial gene mapping

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INSULIN-dependent (type I) diabetes mellitus (IDDM) follows an autoimmune destruction of the insulin-producing β -cells of the pancreas^{1,2}. Family and population studies indicate that predisposition is probably polygenic³. At least one susceptibility gene lies within the major histocompatibility complex and is closely linked to the genes encoding the class II antigens, HLA-DR and HLA-DQ (refs 3, 4). Fine mapping of susceptibility genes by linkage analysis in families is not feasible because of infrequent recombination (linkage disequilibrium) between the *DR* and *DQ* genes. Recombination events in the past, however, have occurred and generated distinct *DR-DQ* haplotypes, whose frequencies vary between races⁴⁻⁶. DNA sequencing and oligonucleotide dot-blot analysis of class II genes from two race-specific haplotypes indicate that susceptibility to IDDM is closely linked to the *DQA1* locus and suggest that both the *DQB1* (ref. 7) and *DQA1* genes contribute to disease predisposition.

The polymorphic *HLA-DQA1* and *-DQB1* genes encode polypeptides that fold together to form a receptor that specifies T-lymphocyte recognition of self and foreign peptides⁸⁻¹³. DNA sequence analysis of *DQ* and *DR* genes from Caucasian patients with IDDM indicates that susceptibility to the disease correlates with the amino acid at position 57 of the *DQ* β -chain, with Asp at this position associated with resistance to IDDM^{7,14-16}. An exception to this is that the 'Asp 57-negative' *DQ* β -chain encoded by *DR3-DQw2* haplotypes, which are positively associated with IDDM, is also encoded by *DR7-DQw2* haplotypes which are not increased in frequency in Caucasian patients^{3,7,17,18}. This difference might arise because *DR3* and *DR7* haplotypes carry different *DQA1* alleles^{7,18}.

In black subjects, race-specific *DR7* and *DR9* haplotypes that are characterized by a unique *TaqI* restriction fragment-length polymorphism (RFLP) in *DQB1*, called *DQBVIc*, have been

TABLE 1 Major histocompatibility complex class II haplotypes and their associations with IDDM in blacks and Caucasians

Locus	<i>DQB1</i>	<i>DQA1</i>	<i>DRB1</i>	<i>DRB4</i>	<i>DRA</i>	Race	IDDM association
Alleles	<i>DQw2</i>	<i>DR7-DQA1</i>	<i>DR7</i>	<i>DRB4</i>	<i>DR4</i>	Caucasian	neutral
	<i>DQw2</i>	<i>DR4-DQA1*</i>	<i>DR7</i>	<i>DRB4</i>	<i>DR4</i>	black	positive
	<i>DQw9</i>	<i>DR4-DQA1</i>	<i>DR9</i>	<i>DRB3</i>	<i>DR4</i>	Caucasian	neutral
	<i>DQw2*</i>	<i>DR4-DQA1</i>	<i>DR9</i>	<i>DRB4</i>	<i>DR4</i>	black	positive

Major histocompatibility complex haplotypes referred to in the text are named according to their DR serological determinants, which correspond to their *DRB1* polymorphism (shown in bold type). These haplotypes were determined by a combination of serological, RFLP and ASO dot-blot analysis^{6,7,23}. Genes are given in the order in which they exist on chromosome 6.

* Allelic substitutions that map susceptibility to IDDM to both the *DQA1* and *DQB1* genes.

TABLE 2 Frequencies of the *DR4-DQA1* allele and *DR7* haplotypes in black type 1 diabetic patients and control subjects

Specificity	IDDM patients (<i>n</i> = 37) <i>n</i> (%)	Controls (<i>n</i> = 79) <i>n</i> (%)	Relative risk	95% Confidence limits	<i>P</i>
DR7, DR4-DQA1	7 (18.9)	1 (1.3)	12.9	2.6-64.0	<0.005
DR7, DR7-DQA1	5 (13.5)	7 (8.9)	1.6	0.5-5.0	NS
DR4-DQA1	29 (78.4)	10 (12.7)	23	8.6-61.0	<10 ⁻⁶

DR7, DR4-DQA1 and DR7, DR7-DQA1 refer to the number (%) of subjects with the DR7 antigen and the *DR4-DQA1* and *DR7-DQA1* alleles, respectively. The total numbers (%) of subjects with the *DR4-DQA1* allele are given in the third row. The characteristics of the diabetic patients and control subjects, and the methods for DR serological typing for the RFLP studies and for the statistical analysis have been described previously⁶. All DR9- and DR7-positive control and patient subjects who possessed the *DR4-DQA1* allele also possessed the *DQB1* RFLP. The two ASOs used to detect the *DR4-DQA1* and *DR7-DQA1* alleles were similar to two probes described previously²³, and had the following sequences, respectively, 5'-TTCCGACAGATTAGAAGATT-3' and 5'-TCTAAGTCTGTGAACA-3'. Hybridization and washing conditions for dot blots were as previously described⁶, and washing temperatures were 59 °C and 50 °C for *DR4-DQA1* and *DR7-DQA1* ASOs, respectively. Using the previously described *DQA1/DQA2*-specific polymerase chain reaction (PCR) primers²⁴ and conditions recommended for *Taq* polymerase²², the signal from the dot blots was not satisfactory. Two per cent of the primary PCR product, obtained using the original *DQA1/DQA2* primers²⁴, was therefore subjected to a second round of *Taq* polymerase-catalysed amplification²² using one of the original primers, GH26a (identical to GH26 (ref. 24), except that the restriction-site bases were not included) with a third oligonucleotide, DQA27B, 5'-GTAGAGTTGGAGCGTTA-3' (corresponding to codons 79-83 of the *DQA1* gene). NS, Not significant.

described⁶. By contrast with the neutral effects of DR7 and DR9 in Caucasians³, both haplotypes are positively associated with IDDM⁶. In the present study, DNA sequences of the class II alleles present on these black haplotypes were analysed and compared with those from non-predisposing Caucasian DR7 and DR9 haplotypes, to identify any class II gene coding differences.

Genes *DRB1*, *DRB4*, *DQA1* and *DQB1* from a black patient, who was homozygous for DR7 and the *DQB1* RFLP, were cloned and sequenced. Analysis of the DNA sequences corresponding to the N-terminal domains of the *DRβ1*-, *DRβ4*- and *DQB1*-chains established that they were identical to those found on Caucasian DR7 haplotypes^{7,14,19,20}. The only sequence difference detected was in the *DQA1* allele. The black *DQA1* allele was identical to the *DQA1* allele found on Caucasian DR4 haplotypes (*DR4-DQA1*; Table 1)²¹.

The *DRB1*, *DRB4*, *DQA1* and *DQB1* alleles from a black patient homozygous for DR9 and the *DQB1* RFLP were also cloned and sequenced. The black DR9 haplotype contained the same *DQB1* allele (*DQw2*) as the black DR7 haplotype, in contrast to Caucasian DR9 haplotypes, which have the Asp 57-encoding *DQw9* allele⁷. The predicted N-terminal amino-acid sequence encoded by the *DQA1* allele was the same as that encoded by Caucasian DR9 and black DR7 haplotypes,

DR4-DQA1. The *DRB1* and *DRB4* alleles were identical to those on Caucasian DR9 haplotypes (Table 1)²⁰.

To assess the contribution of the *DR4-DQA1* allele to IDDM susceptibility, we used allele-specific oligonucleotide (ASO) dot-blot analysis^{7,22,23}. Thirty-seven black patients with IDDM and 79 racially matched controls were tested (Table 2). Two ASOs were used, one that hybridizes to only the *DR7-DQA1* allele, the other to the *DR4-DQA1* allele (Table 2). The analysis revealed that all DR4-positive and DR9-positive patients and control subjects reacted positively with the *DR4-DQA1* ASO, but were negative for the *DR7-DQA1* ASO. Seven out of 11 DR7-positive patients were positive for the *DR4-DQA1* ASO, compared with only 1 out of 8 DR7-positive controls (relative risk of IDDM, 12.9; *P* < 0.005; Table 2). Conversely, 5 out of 11 DR7-positive patients reacted with the *DR7-DQA1* ASO compared with 7 out of 8 DR7-positive controls (relative risk, 1.6, not statistically significant; Table 2). One patient, who was DR7-homozygous, was positive for both ASOs. Overall, the relative risk for the *DR4-DQA1* allele was 23 (95% confidence limits 8.6-61.0; *P* < 10⁻⁶).

The identification of common DR polymorphisms in linkage disequilibrium with a rare combination of *DQA1* and *DQB1* alleles (Table 1) indicates that susceptibility to IDDM maps

FIG. 1 Arrangement of expressed major histocompatibility complex class II genes, *HLA-DR* and *DQ*, on chromosome 6.

centromeric to the DR subregion (Fig. 1). Substitution of the *DR4-DQA1* allele for the *DR7-DQA1* allele on black DR7 haplotypes correlates with a change in predisposition to IDDM from neutral to positive. Furthermore, substitution of the *DQw2-DQB1* allele for the *DQw9* allele on black DR9 haplotypes correlates with a change from neutral to positive. These findings indicate that a particular combination of *DQA1* and *DQB1* alleles is positively associated with IDDM. Although susceptibility may be influenced by other major histocompatibility complex genes, our data suggest that the conformation of the DQ molecules contributes to development of IDDM. □

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Two functionally different regions in Fos are required for the sequence-specific DNA interaction of the Fos/Jun protein complex

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THE products of the cellular and retroviral *fos* genes associate with other nuclear proteins^{1,2}, among them the transcription factor AP1/Jun (see ref. 3 for a review). The Fos/Jun complex binds to a specific symmetrical DNA recognition sequence³⁻⁹ (termed TRE), thus stimulating transcription of the respective gene^{3,9-12}. Here, we show that two distinct regions in Fos are required for the formation of a Fos/Jun/TRE complex. These are the leucine zipper¹³, involved in the association with Jun¹⁴, and a directly adjacent basic region. Specific amino-acid substitutions in this basic, presumably α -helical, region abolish the interaction of Fos/Jun with the TRE but not the association of the two proteins. The functionally crucial amino acids are located in a region of Fos which is structurally similar to the putative DNA-binding sites in Jun and in the yeast transcriptional activator GCN4 (refs 15 and 16).

As a first step in investigating the role of Fos in the formation of a Fos/Jun/TRE complex we attempted to identify by site-directed mutagenesis those sequences in Fos that are crucial for complex formation. We concentrated this investigation on the

middle region of Fos, which evolutionarily is the most highly conserved¹⁷ and is crucial for the induction of transformation¹⁸ and transactivation¹⁰. Two long α -helical regions are predicted in this region (amino acids 139-156 and 163-202)¹⁵. We generated Fos proteins that carried mutations in the putative second helix which forms a leucine zipper¹³ with Jun¹⁴ and various mutants altered in the first helix by exchanging single, pairs or triplets of basic amino acids either with other basic (Arg $\rightarrow$ Lys; Lys $\rightarrow$ Arg) or with neutral (Arg, Lys $\rightarrow$ Gln) amino acids (Fig. 1). We also altered the non-polar sequence Met-Ala-Ala-Ala.

All mutants were analysed for ability to complex with Jun: ³⁵S-labelled, *in vitro*-translated Jun was incubated with unlabelled Fos, followed by immunoprecipitation with Fos-specific antibodies¹⁴. As previously shown¹⁴, binding was either reduced or abolished in several Fos proteins with a mutant leucine-repeat motif (for example, L2, FA183ins, L3-4-5). In contrast, alterations in the basic region did not affect binding to Jun (data not shown).

Next we analysed all mutant Fos proteins in a gel retardation assay using a synthetic oligonucleotide (termed ap1) containing a TRE. Figure 2 (upper panel, lanes 1-4) shows that in the assay conditions used, neither *in vitro*-translated Fos nor Jun shows any significant affinity for the ap1 oligonucleotide. But incubation of the two proteins before addition to the DNA results in a strong shift, indicating that Fos and Jun are binding cooperatively. The specificity of this interaction was demonstrated in competition experiments, in which a >1,000-fold increase in concentration of a random oligonucleotide compared with the specific ap1 oligonucleotide was necessary to abolish the band shift (data not shown). As expected, the ability of the leucine-repeat mutants (L3, L4, L2, L3-4-5, FA183ins) to form a stable Fos/Jun/DNA complex (Fig. 2) correlated well with their ability to associate with Jun¹⁴: the substitution of Leu 179 (L3), Leu 186 (L4) or Leu 172 (L2) either did not affect (L3) or it decreased (L4; L2) the Fos/Jun/DNA complex formation, whereas the triple leucine mutants L3-4-5 and the insertion mutant FA183ins, in which the orientation of leucine residues on one side of the helix is impaired, did not allow complex formation to any significant extent. These data indicate that the association of Fos and Jun through the leucine zipper is required for their interaction with the AP1 DNA-recognition sequence.

Gel retardation analysis of the other mutant Fos proteins revealed a second functionally unrelated domain in Fos that is involved in Fos/Jun/DNA complex formation. This domain is the basic, presumably α -helical structure that is directly adjacent to the leucine repeat, as indicated by the reduced ability of mutant D9 and the failure of D3, D15, D19ins, D4 and D16 to

FIG. 1 Structure of the mutant Fos proteins and the parental E300 used in this study. Substituted amino acids are marked by double underlining. Numbers below the E300 sequence designate amino-acid positions in cellular and viral Fos proteins. The E300 Fos protein used in this study is a FBJ/FBR-MuSV hybrid, extending from amino acid 1-313 (ref. 14). The one-letter amino-acid code is used.

METHODS. Oligonucleotide-directed mutagenesis was by the gapped duplex DNA method based on pMa/5-8 and pMc/5-8 vector systems, as previously described¹⁴. Mutagenic oligonucleotides used were (numbers refer to nucleotide positions in FBR-MuSV²⁵): D6: (2,369) 5'CTCTCGGATTGCT-GTTTCACTTGA (2,344); D8: (2,389) 5'GCAGCAT-CTGATTCTGTTCTCTTCG (2,364); D9: (2,389) 5'GCAG-CATCTGATTCCGTTCTC (2,368); D13: (2,380) 5'CT-TATTCGGTTCTTTTGTATTCTCCGT (2,353); D15: (2,399) 5'GGCACTTGGATGAAGACAGCTTATTCCGTTCT (2,369); D16: (2,410) 5'CCTCCGATTCTTGCACCTGGCTGCAGCC (2,383); D17: (2,421) 5'GTCAGCTCCTCTTCTTATTCGGCAC (2,395); D19ins: (2,400)

	Basic region	Leucine repeat
E 300	...KRRIRRRERNKMAAAKCRNRRRELTD	LQAETDQLDEKSAQLQTEIANLLKEKEKLEF...
	139 150	165 172 179 186 193
Mutations in basic region		Mutations in leucine repeat
D3	KRRIRRRERNKMA...	L2 ...DQVED...
D6	KQIRRRERNKMA...	L3 ...SAVQT...
D8	KRRIRRRERNKMA...	L4 ...ANALK...
D9	KRRIRRRERNKMA...	L3-4-5 ...SAVQTEIANALKEKEKLEF...
D13	KRRIRRRERNKMA...	FA183ins ...TEFAIA...
D15	...NKLSSSKC...	
D19ins	...KMAAAAKC...	
D4	...AAQCONQOQL...	
D16	...AARCKNR...	
D17	...RNKKKEL...	

5'CGGCACTTGGCGGCGCTGCAGCCATC (2,374). Construction of the leucine repeat mutants and of D3 and D4 has been described elsewhere^{10,14}.

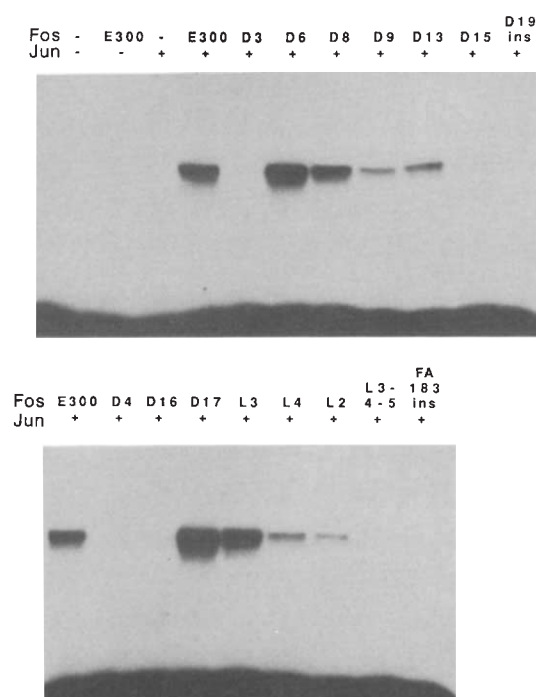


FIG. 2 Gel retardation analysis of complex formation between Fos/Jun and the AP1 DNA-recognition sequence. *In vitro*-translated Fos and Jun proteins were allowed to form a complex as previously described¹⁴ and then incubated with a ³²P-end-labelled double-stranded oligonucleotide (upper strand: 5'AAG-CATGAGTCAGACAC) containing an AP1 recognition sequence. DNA-protein complexes and uncomplexed oligonucleotides were separated on non-denaturing polyacrylamide gels. Protein-DNA complexes were formed in the absence of Fos (E300) or Jun proteins or after incubation of E300 or mutant E300 proteins with Jun. The structure of E300 mutants is shown in Fig. 1. METHODS. E300, fos mutants and c-jun complementary DNA²⁶ were transcribed *in vitro* and translated in reticulocyte lysate¹⁴. Binding reactions were performed by preincubating 5 µl Fos- and 5 µl Jun-containing *in vitro* translation mixtures with 1.5 µg ml⁻¹ poly(dI-dC) in buffer containing 10 mM HEPES, pH 7.9, 60 mM KCl, 4% Ficoll, 1 mM EDTA and 1 mM dithiothreitol for 60 min on ice. Two femtomoles of double-stranded oligonucleotide ³²P-labelled by polynucleotide kinase were added and incubation continued for 45 min at room temperature. The reaction mixtures were separated on 4% polyacrylamide gels at 10 V per cm²⁷.

form a Fos/Jun/DNA complex (Fig. 2). Three aspects of these results deserve particular attention. First, there is no correlation between the elimination of positive charges and the impairment of binding. D6 and D8 (three and two basic amino acids replaced with glutamine, respectively) showed either no change or only a small reduction in their complex-forming potential. In contrast, the mutants having basic for basic amino-acid substitutions (D16) or an altered hydrophobic stretch (D15, D19ins) completely lost their complex-forming potential. Second, the results could indicate that specific interactions with DNA occur in this Fos domain. Although the alteration of certain pairs or triplets of amino acids (D6, D7) did not affect DNA complex-forming ability, the substitution of a single amino acid in D9 or the double mutation in D16 led to a reduction or the abrogation of DNA complex formation. Third, those sequences identified here as being crucial for the formation of a stable Fos/Jun/DNA complex are identical with those most highly conserved between Fos and Jun, namely the putative DNA-binding site in Jun¹⁶ (Fig. 3).

Others have described a cooperative binding of Fos and Jun^{19,20} and a crucial function for the leucine-repeat structure in Fos in the formation of a Fos/Jun/TRE complex^{21,22}. In one study²¹, a single Fos construct containing a mutation outside the leucine repeat structure (Arg-Arg to Glu-Glu at positions 158–159) was found to have no complex-forming potential, a result differing from ours with mutant D17. This may implicate this region in DNA binding as the structures of both mutant proteins seem to be similar, according to structural predictions using two different algorithms^{23,24}.

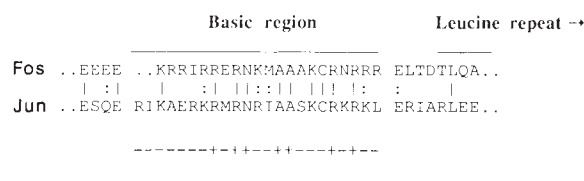


FIG. 3 Region of partial homology between the basic putative α -helices in Fos and Jun. Vertical lines between Fos and Jun sequences indicate identical amino acids; functionally similar residues are depicted by dots. Amino acids that are conserved (+) or not conserved (–) in the basic regions of Fos, Jun and GCN4 are indicated below the sequences. The region in Fos containing amino acids crucial for DNA binding is marked by a line at the bottom of the figure.

On the basis of the available data, we propose a model in which Fos and Jun form a protein complex through the leucine zipper and each constituent interacts with one half-site of the palindromic DNA sequence. This hypothesis is in agreement with the symmetrical nature of the TRE: similar amino-acid sequences in Fos and Jun would interact with similar DNA sequences. The model is also supported by the observation that the DNA sequences directly flanking the TRE (5'...TGA^C/G^TCA...) have no influence on the formation of a stable Fos/Jun/DNA complex (our unpublished results), practically excluding any sequence-specific interaction outside the AP1-recognition sequence. But as direct Fos protein-DNA contacts have been demonstrated by ultraviolet cross-linking experiments⁶, the TRE sequences seem to be the only possible site for a specific interaction of Fos with DNA. A final evaluation of the Fos/Jun/DNA complex will require further mutagenesis, footprint and ultraviolet cross-linking experiments. □

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The generation of mature T cells requires interaction of the $\alpha\beta$ T-cell receptor with major histocompatibility antigens

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THE T-cell repertoire within an individual is biased to recognize antigen in the context of self major histocompatibility complex (MHC) antigens. This is thought to depend on a process of positive selection during development. Support for this notion has recently been obtained in experiments using transgenic mice bearing genes for T-cell receptors (TCR) of defined specificity: T cells expressing the introduced genes form the main part of the mature T-cell population only in mice that express the appropriate MHC product^{1,2}. We have now extended these observations using TCR transgenic mice homozygous for the severe combined immunodeficiency (SCID) mutation which are defective in the rearrangement of both TCR and immunoglobulin genes. In this case mature thymocytes develop only in transgenic mice that express the MHC product which restricts the specificity of the transgenic TCR. This shows that the interaction of the $\alpha\beta$ TCR with thymic MHC antigen is essential for the development of mature T cells. Furthermore, the peripheral lymph nodes of such mice are underdeveloped, suggesting that the peripheral expansion of mature T cells may require interactions with other lymphocytes expressing a range of receptors.

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To produce mice which have only a very limited choice of TCRs we introduced the SCID mutation³ into our $\alpha\beta$ TCR transgenic mice. Mice homozygous for this mutation are grossly defective in productive rearrangements of immunoglobulin (Ig) and TCR genes⁴. We crossed the $\alpha\beta$ TCR transgenic mouse line TG71⁵ with C.B-17/Icr SCID mice and transgenic mice of the F₁ generation were further backcrossed to SCID mice. Mice were typed SCID/SCID by quantitating serum immunoglobulin levels. Because the TG71 line is H-2^b and the C.B-17/Icr line is H-2^d homozygous, H-2^{b/d} heterozygous as well as H-2^d homozygous $\alpha\beta$ -transgenic SCID mice were obtained in the backcrosses. In initial experiments we compared the thymus of the SCID and transgenic SCID mice: the thymus of the former contained on average 2×10^6 thymocytes whereas that of the latter contained more than 5×10^7 thymocytes irrespective of the MHC haplotype. Although thymocytes from both types of mice were Thy-1-positive, only those of the transgenic SCID mice expressed CD4 and CD8 molecules (Fig. 1). (The weak staining of thymocytes from SCID mice with CD4 antibodies was probably due to nonspecific binding of this conjugate to CD4⁻ lymphocytes because it was not observed with another CD4 antibody.) These results imply that the maturation of CD4⁺8⁺ thymocytes from CD4⁻8⁻ precursors⁶ requires the productive rearrangement of TCR DNA segments. Crosses with mice expressing only the α - or β -transgene are required to determine which TCR gene segments are necessary and sufficient for this step. These data indicate that the only defect of SCID mice is the defective rearrangement mechanism.

In further experiments we compared the cellular composition of the thymus from female H-2^{b/d} and H-2^d $\alpha\beta$ -transgenic SCID mice. Because the transgenic TCR would essentially be the only receptor which could be used by the animals, one might expect that the H-2^{b/d}, but not the H-2^d animals, would contain CD4⁺8⁺ thymocytes, if an interaction of the TCR with MHC antigens was essential for the generation of mature T cells. Double staining with CD4 and CD8 antibodies showed that this was indeed the case: the thymus of the H-2^{b/d} transgenic SCID mice contained CD4⁺8⁻, CD4⁺8⁺, CD4⁻8⁺ but not CD4⁻8⁻ cells whereas that of the H-2^d transgenic SCID mice contained

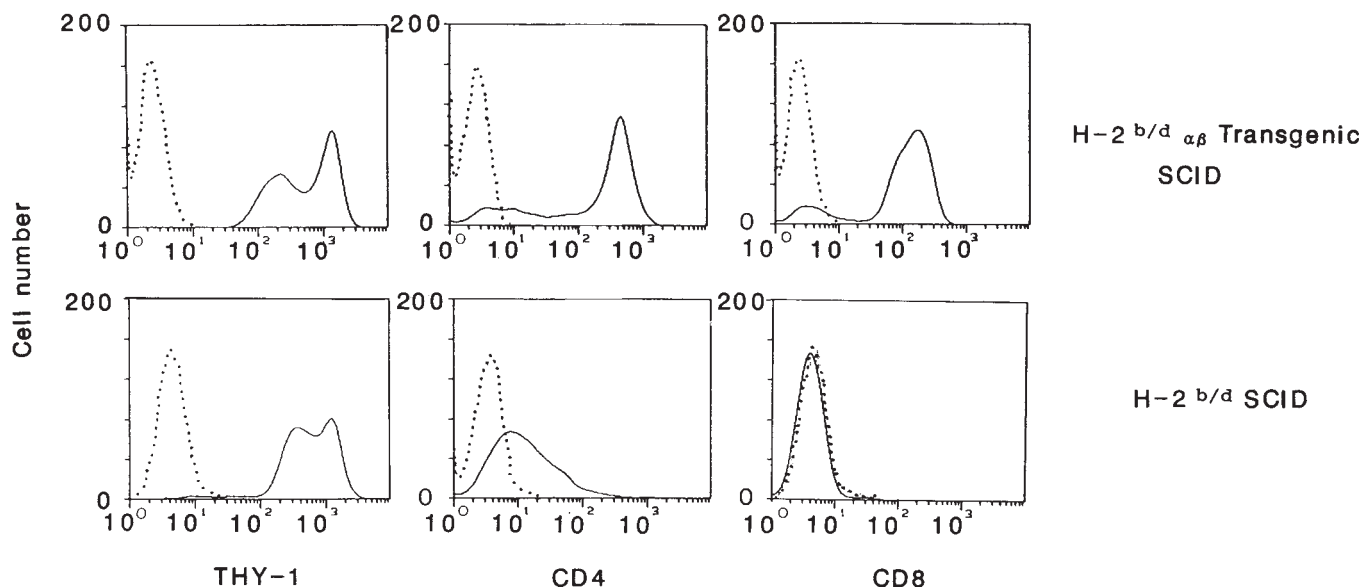


FIG. 1 Generation of CD4⁺ and CD8⁺ thymocytes in $\alpha\beta$ -transgenic SCID mice. Thymocytes from female H-2^{b/d} $\alpha\beta$ -transgenic SCID (upper panels) and H-2^{b/d} SCID mice (lower panels) were analysed for the expression of Thy-1, CD4 and CD8. Cell numbers are represented on the vertical axis and fluorescence, on a logarithmic scale, on the horizontal axis. The low level of CD4 staining of thymocytes from SCID mice is probably due to the non-specific binding of this reagent to large lymphoblasts which predominate

in the SCID thymus.

METHODS. Single staining of cells involved either incubation with a directly labelled antibody (CD4 or CD8) or a first stage antibody (anti-Thy-1, F23.1 or T3.70) followed by a second stage FITC conjugated (Fab)₂ sheep antimouse immunoglobulin (FITC-S α Mlg, Silenus). All incubations were for 20 min on ice with two washes between the steps and before analysis.

CD4⁻8⁻ and CD4⁺8⁺ but neither CD4⁻8⁺ nor CD4⁺8⁻ cells (Fig. 2). Staining with the F23.1 (not shown) and T3.70 monoclonal antibodies detecting the transgenic β and α TCR chains, respectively⁷ indicated that in the various transgenic SCID mice practically all thymocytes expressed the transgenic receptor. The H-2^{b/d} $\alpha\beta$ -transgenic SCID mice contained two populations of cells expressing high levels of the transgenic $\alpha\beta$ -receptor, namely CD4⁻8⁻ and CD4⁻8⁺ thymocytes. The only population with a high density of TCRs was the CD4⁻8⁻ population in H-2^d transgenic SCID mice. Both types of animals contained CD4⁺8⁺ thymocytes expressing lower levels of the transgenic TCR^{1,2}. Similar results were obtained in additional two H-2^{b/d} and four H-2^d $\alpha\beta$ -transgenic SCID mice. Thus, by contrast with $\alpha\beta$ -transgenic H-2^d mice, the thymus of H-2^d $\alpha\beta$ -transgenic SCID mice does not contain significant numbers of mature CD4⁺8⁻ or CD4⁻8⁺ thymocytes. This indicates that the generation of mature, single, positive thymocytes requires a TCR-

MHC interaction and that positive selection does not simply reflect the preferential expansion of some mature T cells.

The size of the thymuses in the $\alpha\beta$ -transgenic SCID mice was almost normal whereas the peripheral lymphoid tissue in all transgenic SCID mice was clearly underdeveloped: the lymph nodes of these mice contained on average only 10 % of lymphocytes when compared with $\alpha\beta$ -transgenic mice kept under identical conditions. Double staining with various antibodies showed that the lymphocytes from only H-2^{b/d} mice contained significant numbers of CD4⁺8⁺ T cells which stained with both F23.1 and T3.70 antibodies. H-2^d transgenic SCID mice contained very few CD4⁺8⁺ cells and most of these did not express the transgenic α -chain. Both types of animals contained over 20 % of CD4⁻8⁻ lymphocytes expressing the transgenic receptor. Finally, in H-2^{b/d} as well as H-2^{d/d} mice, ~30% of CD4⁺8⁻ T cells were detected which expressed mostly the transgenic β - but not the transgenic α -chain (Fig. 3). Immunoglobulin-

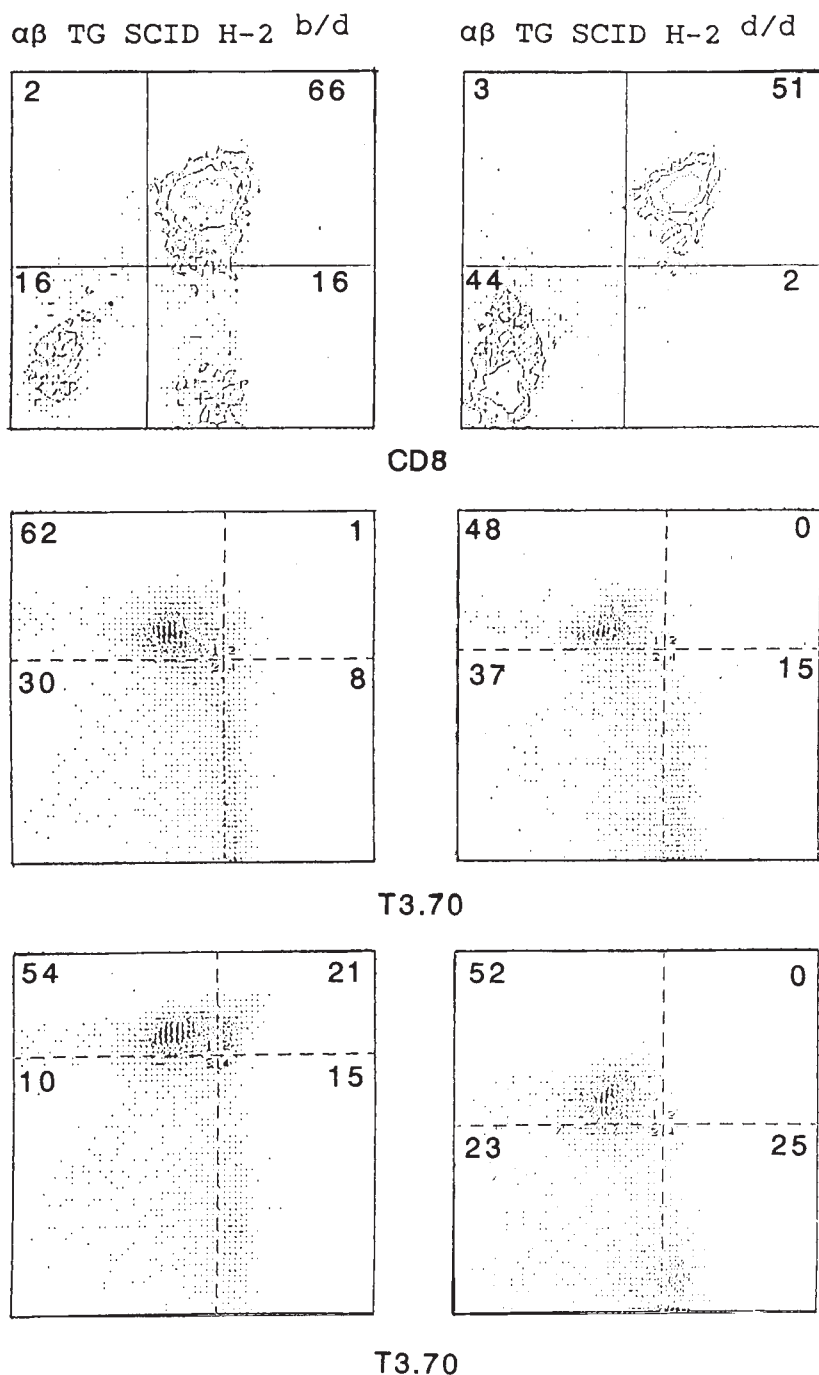


FIG. 2 Thymocyte subpopulations defined by CD4, CD8 and T3.70 antibodies. Thymocytes from female H-2^{b/d} and H-2^{d/d} $\alpha\beta$ -transgenic SCID mice were double stained with CD4/CD8, CD4/T3.70 and CD8/T3.70 antibodies. Numbers in quadrants give the percentage of cells in the quadrants. The conditions of staining and the antibodies have been described previously^{1,2}.

METHODS. Double labelling of cells involved a first stage incubation with either F23.1 or T3.70 followed by a second incubation with FITC-S α Mlg. Potentially free sites for binding of immunoglobulin on the fluoresceinated reagent were saturated by a third incubation with normal mouse serum (2% v/v). The cells were then incubated with either PE-anti CD4 or biotinylated anti-CD8 antibodies. Cells incubated with the latter required a final stage of PE-streptavidin (Becton Dickinson). All incubations were for 20 min on ice with two washes between each step and before analysis. All analyses were performed on the FACScan flow cytometer (Becton Dickinson). Single staining required the analysis of 5,000 cells per sample; double staining required 10,000 cells per sample. Dead cells were excluded from the analyses on the basis of low, forward and sideways, light-scattering properties. All graphs were produced from the use of FACScan research software except the contour plots which utilized a CONSORT-30 program.

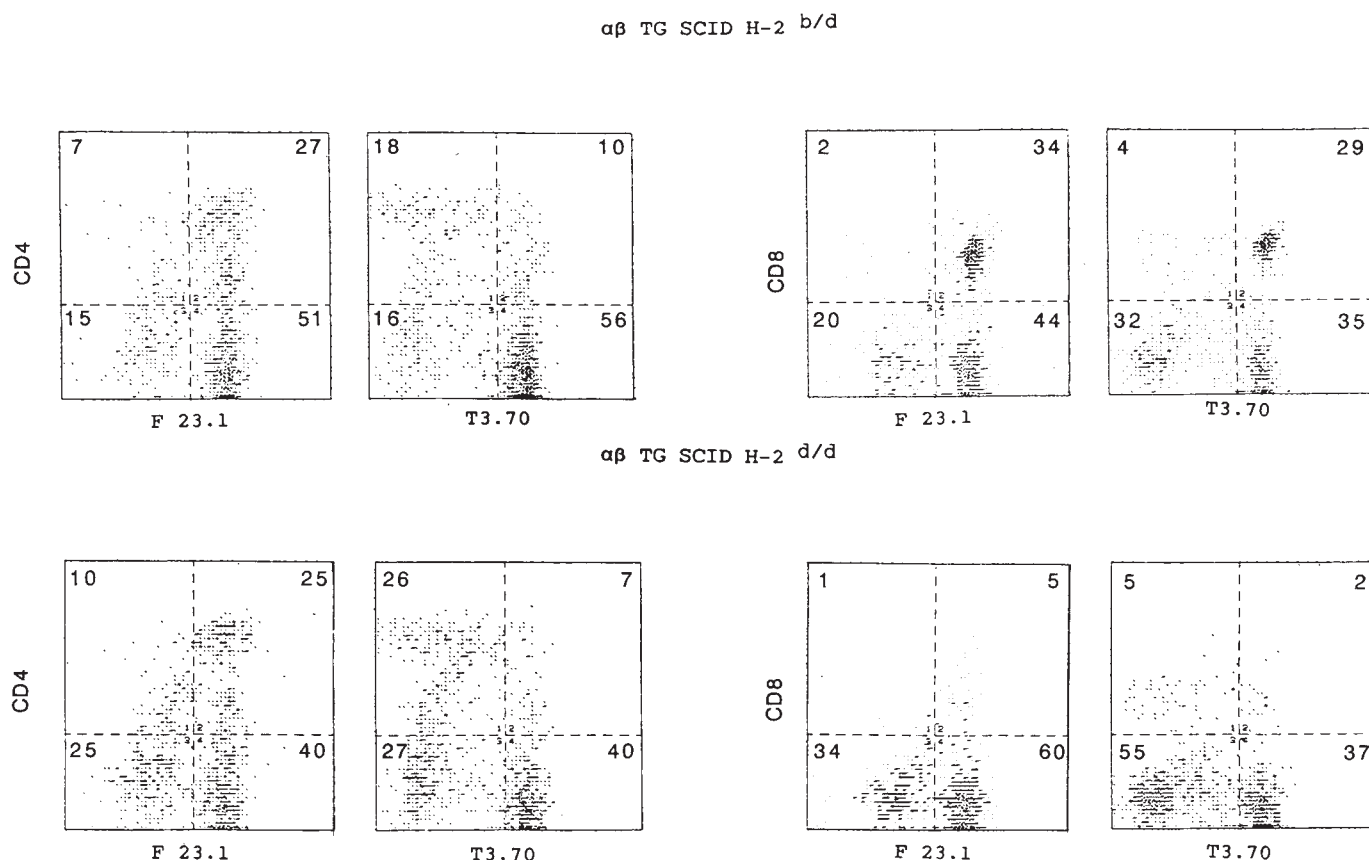


FIG 3 Lymphocyte subpopulations defined by CD4, CD8, F23.1, and T3.70 antibodies. Lymphocytes from female H-2^{d/d} and H-2^{b/d} $\alpha\beta$ -transgenic SCID mice were double stained with CD4/F23.1, CD4/T3.70, CD8/F23.1 and

CD8/T3.70 antibodies. Numbers in the quadrants give the percentage of lymphocytes in the quadrants. Conditions of staining and the antibodies have been described previously^{1,2}.

positive cells were not detected in any of the lymphocytes. These data indicate that productive rearrangements are made infrequently in SCID mice. In non-transgenic SCID mice this is not often observed because the formation of a receptor-bearing lymphocyte requires several consecutive productive rearrangements in a given lineage. In the transgenic SCID mice, productive α -gene rearrangements are much easier to detect because the endogenous α -chain can pair with the transgenic β -chain, the formation of which would require two productive rearrangements in non-transgenic SCID mice. This is probably the reason why we see T cells expressing endogenous α TCR chains but cannot detect significant numbers of B cells, which again would require multiple productive rearrangements of endogenous immunoglobulin receptor gene segments. Because we cannot detect significant numbers of CD4⁺8⁺ or CD4⁺8⁻ thymocytes expressing endogenous α -chains in the H-2^d $\alpha\beta$ -transgenic SCID mice, we conclude that very few cells of these phenotypes are generated. But when they are and express a selectable receptor, they leave the thymus and accumulate in the peripheral lymphoid tissue.

This is in marked contrast to CD4⁺8⁻ cells expressing high levels of TCR. These cells are at least as frequent in the thymus and periphery in H-2^d as in H-2^{b/d} $\alpha\beta$ -transgenic SCID mice even though they express the same $\alpha\beta$ -transgenic receptor. Thus the generation of $\alpha\beta$ TCR positive CD4⁺8⁻ peripheral T cells does not require positive selection by restricting D^b MHC antigens.

As $\alpha\beta$ -T-cells are produced in the SCID mice in the absence

of detectable $\gamma\delta$ cells it seems that the latter do not play an essential part in the development of CD4⁺8⁺, CD4⁺8⁻ or CD4⁺8⁺ T cells. This does not exclude $\gamma\delta$ -cells from being essential for the formation of cells expressing rearranged TCR β -genes because our mice contain already rearranged β -genes.

Finally, the underdeveloped tissue of secondary lymphoid organs in H-2^{d/b} $\alpha\beta$ -transgenic SCID mice deserves some comment: in these mice CD4⁺8⁺ cells are continuously positively selected in thymus yet the absolute numbers of peripheral lymphocytes are small. This suggests that the formation of peripheral lymphoid tissue requires immunoglobulin positive lymphocytes as well as CD4⁺8⁻ T cells with a diverse range of receptors. Thus, antigenic stimulation and interaction of various lymphocyte subsets may be an important factor regulating the size of lymphoid tissue. □

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Phenol stabilizes more helix in a new symmetrical zinc insulin hexamer

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SINCE insulin was first shown by Scott¹ to crystallize in the presence of zinc ions in 1934, a variety of Zn-containing insulin crystals have been grown^{2,3}. The structures of insulin in the related rhombohedral crystals of 2Zn-insulin and 4Zn-insulin have been solved^{4,5} and reveal that the molecule is a hexamer, organized as three dimers, each containing a 2-fold symmetry axis and held together by Zn ions. In 2Zn-insulin the hexamer is nearly symmetrical with the two axial Zn ions and the two molecules of the dimer related closely by a local 2-fold axis. But in 4Zn-insulin the two molecules in the dimer differ remarkably, creating an asymmetric 4Zn-hexamer in which one trimer is essentially equivalent to that in 2Zn-insulin and the other is different by virtue of an additional stretch of N-terminal helix between residues B1 and B8 (refs 6, 7). We report here the structure of a new symmetrical hexamer, in which all six molecules have the B1–B8 helix seen in 4Zn-insulin. Phenol molecules, found bonding specifically to each molecule, evidently stabilize this new helical conformation.

The existence of a symmetrical hexamer in which all six insulin molecules have a helical conformation from B1–B20 has been predicted^{8,9} and the capacity of phenol to promote this helix in solution has been observed¹⁰. Monoclinic crystals of hexameric insulin grown in the presence of phenol, added as a preservative, have long been known² and it was from these that the new hexamer was discovered. The space group is *P2*₁ and the crystals contain a complete hexamer in the asymmetric unit (Table 1). The chemical analysis of these crystals showed that they contained two Zn ions per insulin hexamer², which suggested they were related in a simple manner to that of the rhombohedral 2Zn-insulin hexamer.

A full X-ray analysis of these crystals was originally under-

taken to investigate the loss of 3-fold symmetry in the hexamer and its effect upon the conformation of each of the monomers. Early experiments to prepare heavy-atom derivatives had limited success (J. Dargay, D. Hodgkin and G.G.D., unpublished observations). The structure was determined by molecular replacement, using the accurate 2Zn-insulin coordinates¹¹ as an initial model, and refined^{12,13}. During the refinement it became clear that the hexamer in the crystal was different from the assumed 2Zn-insulin model, primarily at residues B1–B8. Inspection of the difference Fourier maps revealed electron density tracing a helix from B9–B1 in all six molecules, reminiscent of that seen in one trimer of the rhombohedral 4Zn-insulin hexamer (see Fig. 1). Consequently, a new hexamer was constructed using six monomers with properly oriented α -helices from B1–B20.

As the refinement converged, a large region of strong electron density, obviously not water, was identified next to each of the B5 His side-chains. Continued refinement with the 1.8 Å data resulted in a map in which the phenol molecules were mostly well resolved and the positions of their hydroxyl groups clearly indicated (see Fig. 2). Each of the six phenol molecules donates a hydrogen bond to the A6 Cys carbonyl oxygen and accepts a hydrogen bond from the A11 Cys amide nitrogen. The phenol molecules sit in the cavity created by the A-chain residues from one dimer and the B1–B8 helical structure from the adjacent dimer. This allows the B5 His to link the dimers through van der Waal's contacts with the phenol molecule; in 4Zn-insulin, the same effect is achieved by the off-axial Zn ions. As shown in Fig. 1 there is a clear progression in the amount of helix present between residues B1 and B20 in the hexamers in rhombohedral 2Zn-insulin, 4Zn-insulin and monoclinic insulin crystals. These hexamers have been designated respectively 'T₆', 'T₃/R₃' and 'R₆', as described in Fig. 1.

The finding that phenol binds and stabilizes helix at B1–B8 in the insulin hexamer is a reminder of how complex interactions between ligands and proteins can be. It also raises the question of how the Zn-insulin hexamers stored in the β -cell are constructed: the high concentrations of peptides, Ca²⁺, Zn²⁺ and counterions could all influence the conformation at B1–B8. Moreover, the switch from extended sheet to helix is reminiscent of structural transitions observed in signalling and allosteric mechanisms, often associated with Zn²⁺ or Ca²⁺ (ref. 14).

The similarity between phenol and tyrosine raises the possibility that a tyrosyl side-chain from the insulin receptor stabilizes the B-chain N-terminal helix in the course of generating a signal

TABLE 1 Crystallographic and structural data

Hexamer type	Space group	No. molecules per asymmetric unit	Cell parameters	R_{cryst}^*	Resolution	Approximate point symmetry in hexamer	No. Zn sites	Chemical species associated with Zn	Limits of B-helix
2Zn-insulin (T ₆)	<i>R</i> 3	2	$a_h = 82.5$, $b_h = 82.5$, $c_h = 34.0$, $\gamma = 120^\circ$	0.154	1.5 Å	32	2	H ₂ O	B9–B20 M1‡ B9–B20 M2
4Zn-insulin (T ₃ /R ₃)	<i>R</i> 3	2	$a_h = 80.7$, $b_h = 80.7$, $c_h = 37.6$, $\gamma = 120^\circ$	0.18	1.5 Å	3	5	Cl, H ₂ O	B9–B20 M1 B1–B20 M2
2Zn-insulin phenol (R ₆)	<i>P</i> 2 ₁	6	$a = 61.36$, $b = 61.71$, $c = 47.95$, $\beta = 110.8$	0.22	1.8 Å†	32	2	Cl, H ₂ O	B1–B20 M1 B1–B20 M2 B1–B20 M3 B1–B20 M4 B1–B20 M5 B1–B20 M6

* $R_{\text{cryst}} = \sum ||F_o| - |F_c|| / \sum |F_o|$ where $|F_o|$ and $|F_c|$ are the observed and calculated structure amplitudes respectively (all data included).

† Diffraction data collected on a diffractometer extend to 2.5 Å (15,785 reflections); data collected with synchrotron radiation extend to 1.8 Å (30,841 reflections). The molecular replacement and initial refinement calculations were carried out on the diffractometer data. Refinement of the atomic positions and thermal parameters were carried out on a combined diffractometer and synchrotron radiation data set ($R_{\text{merge}} = 0.06$). The usual Fourier and fast Fourier least squares minimization methods were used in which the calculated shifts were restrained by the requirements of peptide geometry^{12,13}. All 2,430 protein atoms in the asymmetric unit have been located and refined; the six phenol molecules and 421 water molecules have also been identified and included in the refinement calculations. The R_{cryst} on all data is 0.22. The r.m.s. deviation from ideal bond lengths is 0.03 Å; accuracy of the well defined atomic positions is estimated to be about 0.2 Å. The atomic parameters will be deposited in the Brookhaven Data Bank.

‡ M1–M6 represent the insulin molecules in the asymmetric unit.

FIG. 1 Arrangement of the three monomers around the upper (a) and lower (b) Zn ions, and the complete hexamer structures (c) in the 2Zn- and 4Zn-insulin crystals and the 2Zn-insulin (phenol) crystals. C α positions are shown together with B5 and B10 histidyl side chains involved in Zn coordination. The A-chain is drawn with open bonds, the B-chain B9-B30 is drawn as a single line and the B-chain residues B1-B8 are drawn in heavy lines. The phenol molecules and the off-axial Zn sites in 4Zn-insulin are also shown. The stoichiometry of Zn in 4Zn-insulin is not well defined: when the off-axial sites are fully occupied in molecule 2 (b), there are 4Zn ions per hexamer, but two populations, with alternative B10 His conformations also occur. One of these has Zn bound on the axial site (to B10 His); the other has Zn bound at the off-axial site (B5 His and B10 His). The conformation that does not bind phenol is analogous to an allosteric T state; the extended conformation at B1-B8 is thus called T. By the same analogy, R represents the structure with helix at B1-B8 that binds phenol.

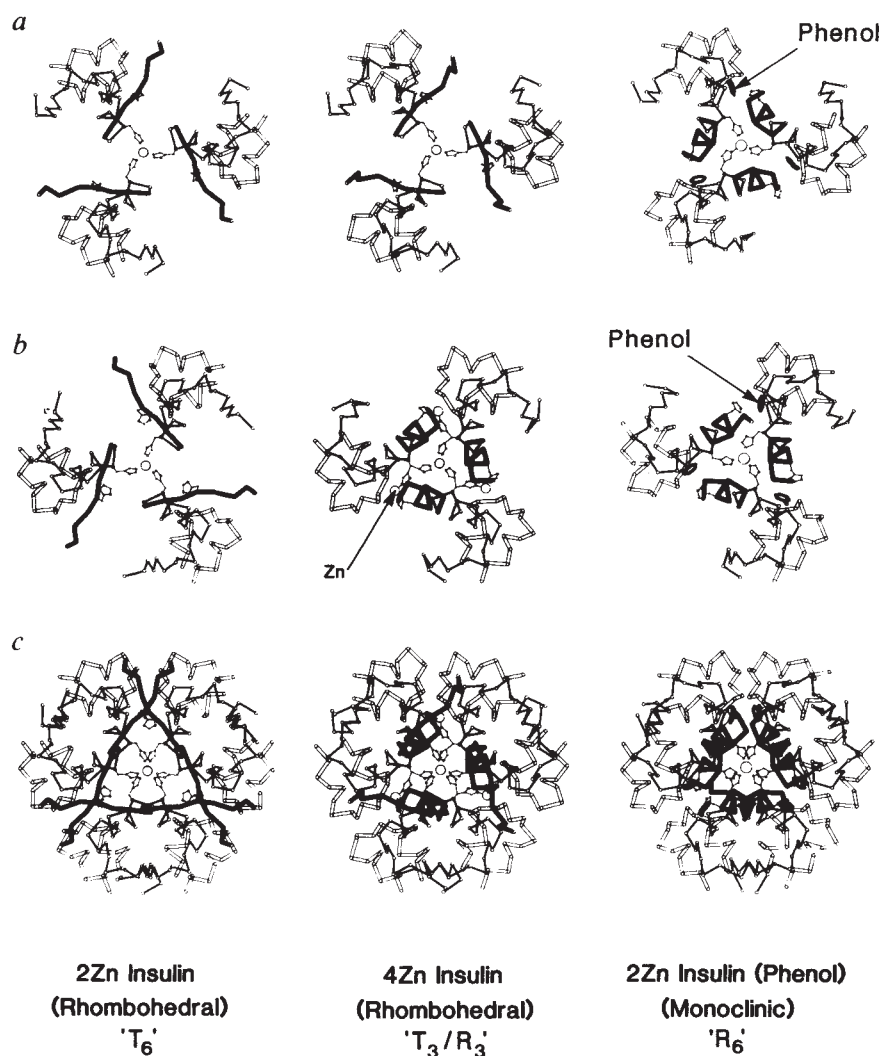
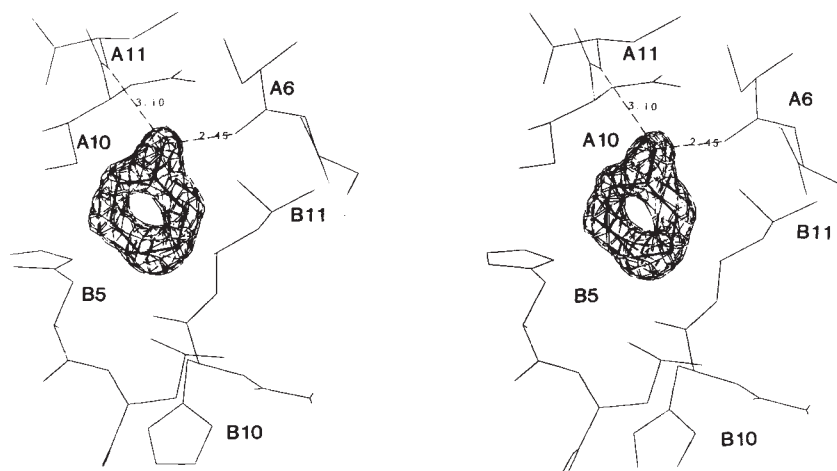


FIG. 2 The electron density of one of the phenol molecules. The OH group is hydrogen-bonded to the carbonyl oxygen and amide nitrogen of the A6 and A11 cysteines respectively, linked in a disulphide bond. The phenol makes van der Waal's contacts with the dimer-dimer interface (Fig. 1). The imidazole B5 group makes a roughly perpendicular approach of 3.4 Å to the phenol molecule. It belongs to an insulin molecule from one dimer; the A-chain residues surrounding the other surfaces of the phenol are from the insulin molecule in the adjacent dimer. Other contacts are not shown.



after hormone binding. Independent studies have shown that phenol binding greatly reduces exchange with solution by the Zn^{2+} ions trapped by the B1-B8 helix extension (A. Wollmer and M. Dunn, personal communication). Several commonly used insulin preparations use phenol or similar molecules as a preservative¹⁵. The protamine (NPH) insulin, for example, contains phenol or cresol at concentrations (0.1–0.25%) high enough to induce the helix structure at B1-B8 observed in the monoclinic insulin crystals. Indeed, the 'R₆' hexamer with six bound *m*-cresol molecules has been recently identified in NPH insulin crystals, helping to explain their extra stability (F. Korber, personal communication). It is conceivable that other phenol-like molecules could also be used to enhance the stability of the hexamer in solution, leading to a range of insulin preparations of increased stability. □

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Biosynthesis of cadmium sulphide quantum semiconductor crystallites

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NANOMETRE-SCALE semiconductor quantum crystallites exhibit size-dependent and discrete excited electronic states which occur at energies higher than the band gap of the corresponding bulk solid^{1–4}. These crystallites are too small to have continuous energy bands, even though a bulk crystal structure is present. The onset of such quantum properties sets a fundamental limit to device miniaturization in microelectronics⁵. Structures with either one, two or all three dimensions on the nanometer scale are of particular interest in solid state physics⁶. We report here our discovery of the biosynthesis of quantum crystallites in yeasts *Candida glabrata* and *Schizosaccharomyces pombe*, cultured in the presence of cadmium salts. Short chelating peptides of general structure (γ -Glu-Cys)_n-Gly control the nucleation and growth of CdS crystallites to peptide-capped intracellular particles of diameter 20 Å. These quantum CdS crystallites are more monodisperse than CdS particles synthesized chemically. X-ray data indicate that, at this small size, the CdS structure differs from that of bulk CdS and tends towards a six-coordinate rock-salt structure.

C. glabrata and *S. pombe* respond to Cd salts by synthesizing γ -glutamyl peptides^{7,8}. Cytoplasmic cadmium ions are sequestered within complexes containing these γ -Glu peptides⁹. Related metal- γ -peptide complexes are found in metal-exposed plants and *Euglena gracilis*^{10–12}. The metal-peptide complexes readily incorporate sulphide ions arising from a Cd-mediated enhancement of cellular sulphide generation^{13–15}. Typical isolates of such cytoplasmic metal-thiolate-peptide complexes can be resolved by gel filtration into clusters varying in sulphide content¹⁴. The Stokes' radius of the metal-thiolate-peptide complex increases in direct proportion to the sulphide/cadmium ratio. Thus, complex size seems to be dictated by the magnitude of the metal-induced sulphide response.

Transmission electron microscopy (TEM) of the Cd- γ -Glu peptide complex (S/Cd=0.7) from *C. glabrata* shows dense aggregates of particles, each having diameters of 20 ± 3 Å and a Stokes' radius of ~ 16 Å. Similar complexes from *S. pombe* (S/Cd=0.6) are ~ 18 Å in diameter. In both cases, the powder X-ray pattern of lyophilized material shows one sharp low-angle peak (Fig. 1), indicating a dense aggregate of near monodisperse, spherical, homogeneous particles. The particle diameters obtained by X-ray diffraction are consistent with those from the electron microscopy.

The Bragg diffraction patterns show clear differences between the biogenic crystallites and the bulk solid. The Cd-S bond is on average shorter in the crystallite by about 3–6%. The diffraction pattern from the *S. pombe* complex is accounted for best by the six-coordinate rock-salt structure; a structure previously reported for CdS only at high pressure¹⁶. The *C. glabrata* complex gives rise to a diffraction pattern consistent with a structure which is intermediate between rock-salt and the naturally occurring zinc-blende four-coordinate structure. Both show an 8 Å coherence length, corresponding to a short network of about four identical Cd-S bonds; surface-bonding changes may contribute to such short lengths in small particles. TEM internal lattice images are not on average observed because of this short

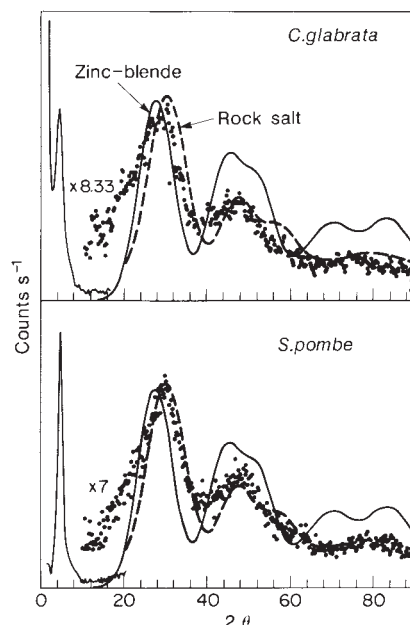


FIG. 1 1.54 Å powder X-ray patterns of desalted lyophilized Cd- γ -Glu peptide complexes from *C. glabrata* (top) and *S. pombe* (bottom). The samples were purified by a combination of ion-exchange and gel-filtration chromatography as described previously^{9,15}. The samples were desalted by chromatography on Sephadex G-25 equilibrated and eluted with deionized water. Desalted samples were dried in X-ray tubes under vacuum. Solid and dashed lines, expected patterns for zinc-blende and rock-salt lattices, respectively, with an 8 Å coherence length. The sharp peak at low angle (4°) represents particle spacing in the dense isolate. The nearest-neighbour distances are 22 Å and 20 Å for isolates from *C. glabrata* and *S. pombe* respectively.

coherence length. The γ -glutamyl peptide coating of the *C. glabrata* 20-Å crystallites consists of approximately 30 peptides of predominantly n_2 and n_2 desGly. The cysteinyl thiolates in the peptides are surface ligands to the core of ~ 85 CdS units.

Both yeasts also produce larger, extracellular CdS particles of uncharacterized coating, whose high apparent molecular weight is indicated by sedimentation at 40,000g and elution in the excluded volume on Sepharose 6B. Particles of diameter 29 ± 5 Å, some of which show zinc-blende internal lattice images (Fig. 2), are shown by electron microscopy. The X-ray Bragg pattern also reveals a zinc-blende structure with an 8 Å coherence length.

The optical band gap of synthetic CdS quantum crystallites increases with decreasing diameter, in agreement with theory¹⁷. Similarly, the lowest excited state of the 20 Å intracellular particles occurs between 300 and 315 nm (Fig. 3), almost 1.5 eV above the bulk band gap. Smaller sulphide-deficient complexes in *S. pombe* have transitions shifted to higher energy. This spectral shift¹⁴ reflects the size variation of the CdS core. Significantly, extracellular zinc-blende CdS particles of diameter ~ 29 Å have a 365 nm transition (Fig. 3) which is similar to that of synthetic CdS crystallites of comparable diameter (Fig. 3, inset).

Approaches to synthesizing quantum crystallite colloids of CdS have used microstructured environments and/or polymeric surfactants to prevent irreversible precipitation of bulk solid¹⁸⁻²³. Quantum crystallites previously isolated and purified, have been capped with small organic groups²⁴, or with polymeric sodium hexametaphosphate²⁰. Only zinc-blende structures have been reported. The narrow, low-angle X-ray peaks (Fig. 1), and the narrow optical transition in the *C. glabrata* particle, imply that these biogenic, short-peptide-capped CdS crystallites are more monodisperse than typical synthetic particles.

Preliminary experiments *in vitro* indicate that these peptides are effective in terminating the growth of crystallites of a specific size. The intracellular crystallites exhibit properties common to small synthetic semiconductor particles such as luminescence at ~ 460 nm and photo-induced electron transfer to methyl viologen. At physiological pH the peptide-coated particles are stable; at pH 3.5, accretion and growth readily occur.

We report here the first demonstration of the biological synthesis of quantum semiconductor crystallites of CdS. Because

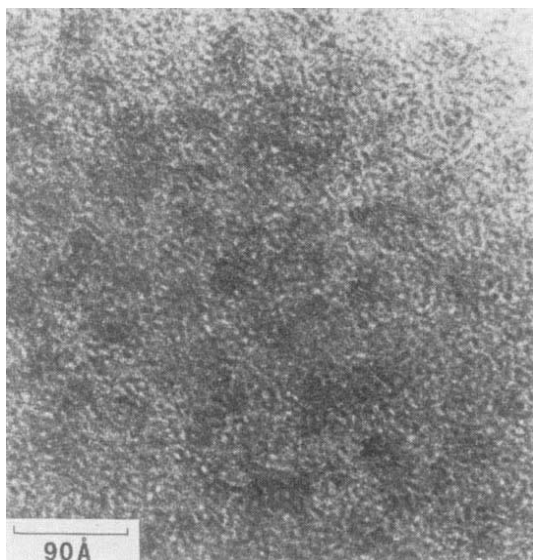


FIG. 2 High-resolution, axial bright-field transmission electron micrograph taken near the Scherzer focus of the *S. pombe* extracellular CdS particle. The sample was dissolved in an aqueous ionic surfactant solution and dried on a thin carbon substrate. Some crystallites show faint images of internal lattice structure when a (111) axis lies in the substrate plane.

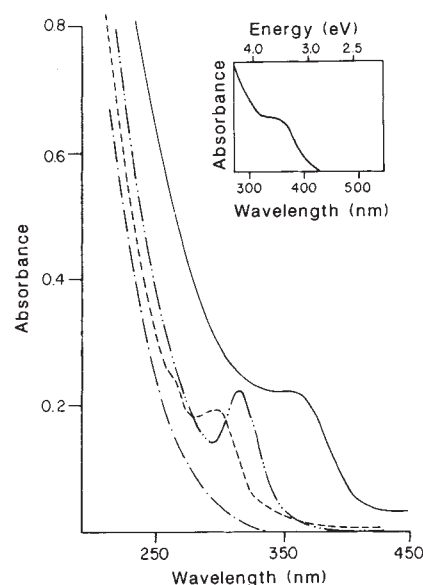


FIG. 3 Optical absorption spectra of the CdS complexes. The samples include peptide-bound CdS particles from *C. glabrata* (—), *S. pombe* (---) and the extracellular CdS particle from *S. pombe* (-.-). The samples contain 4, 8 and 20 μ g Cd²⁺ per ml, respectively. The extracellular CdS complex was isolated by sedimentation at 40,000g and followed by washings. The precipitate was resuspended in 20 mM potassium phosphate, pH 6.5, and concentrated by ultrafiltration (Amicon PM30) followed by filtration (0.2 μ m filter). The 0.2 μ m filtrate was precipitated with 30 mM Mg²⁺ and the final precipitate was resuspended in water and analysed. The (—) line represents the absorption spectrum of *C. glabrata* CdS peptide complex acidified to pH 1.5 to remove H₂S and re-neutralized to pH 7. The inset is the optical absorption spectra of a chemically prepared 30-Å-diameter zinc-blende CdS crystallite described previously⁴.

the dissociation constant of crystalline CdS is extremely small, bioformation of solid CdS sequesters and thus detoxifies intracellular Cd ions. Electron-dense CdS granules have also been observed on the cell surface of the bacterium *Klebsiella aerogenes*²⁵.

Quantum, metal sulphide crystallites have optical absorption, photosynthetic and electron transfer properties that are size-tunable^{1,3}. The peptides used by these yeasts occur quite commonly in nature. It is conceivable that in some organisms metal sulphide crystallites have a physiological role other than detoxification. □

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Chemiluminescence lights up

I. Bronstein and P. McGrath

Chemiluminescent substrates for alkaline phosphatase and β -galactosidase are now available for use in immunoassays and DNA hybridization assays.

ONE of the most fascinating displays of energy release is the phenomenon of luminescence. In the broadest sense, luminescence is the conversion of energy into light. The energy required for the emission of light is generated by the oxidation of a specific substrate. Biological luminescence, or bioluminescence, occurs in organisms such as bacteria, fungi and insects¹. The firefly *Photinus pyralis*, the most familiar example of bioluminescence, generates an incredibly efficient 88 per cent of its energy as luminescence. The mechanism of this reaction involves the adenylation of firefly luciferin by ATP in the presence of the enzyme firefly luciferase. Luciferin adenylate is then oxidized to a peroxide which ultimately decomposes to oxyluciferin, AMP, and CO₂, accompanied by light emission².

The use of firefly bioluminescence in immunoassays has been demonstrated³, but the complexity and instability of the system makes this application impractical. The high background signal arising from ATP contamination further reduces the commercial potential of luciferase, because most bioluminescent assays are based on ATP detection⁴. Several attempts to conjugate the luciferase enzyme to antibodies have failed because of the instability of the constructs; the only successful example of labelling an anti-human antibody with bacterial luciferase is used in a rubella antibody assay⁵. Recently, the gene for luciferase has been cloned⁶, and recombinant firefly luciferase is now on the market.

Chemiluminescent glow

Highly sensitive photomultipliers have been used to show that chemical luminescence, or chemiluminescence, is a universal property of organic substances able to undergo an oxidative reaction sufficiently exothermic to produce an emitting state. (Lightning is an example of chemiluminescence in the gases of the earth's atmosphere.) But initial expectations that chemiluminescence could be adapted readily for use in clinical diagnostics have turned to scepticism. The luminol 5-amino-2,3-dihydrophthalazine-4,4-dione, for example, when utilized as an antibody label allows only subnanomolar detection after activation with hydrogen peroxide, alkali and microperoxidase⁷. Peroxyoxalate chemiluminescence — generated from the reaction of oxalyl chloride, hydrogen peroxide and a

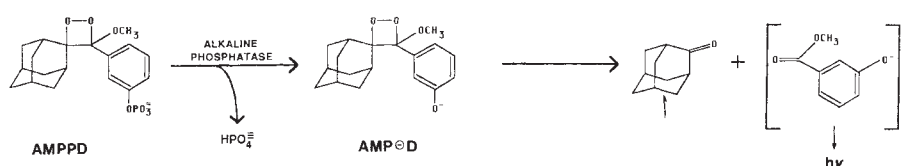


FIG. 1 Adamantyl-1,2-dioxetane phosphate (AMPPD), substrate for alkaline phosphatase, and its mechanism of chemiluminescent decomposition.

fluorescent compound such as anthracene or fluorescein⁸ — shows very high efficiency in organic solvents, but in aqueous environments its quantum yield is considerably reduced⁹. The chemiluminescent signal obtained from these systems is also in the form of a short-lived (microsecond to millisecond) "flash" which requires expensive, highly sensitive photomultipliers for recording in real time.

Despite its difficulties, luminescence offers several advantages over other analytical labelling techniques: high sensitivity, low background, a wide linear range, low cost per test, and no radiation hazards. Systems which provide a chemiluminescent "glow" signal rather than a "flash" also can be detected with simple and inexpensive instruments, such as photodiode-based luminometers, and X-ray and instant photographic films.

Some luminescent labels used in bioassays can be quantified rapidly, and are relatively stable. To date, commercial chemiluminescent systems include acridium esters and enhanced luminols. (*N*-methylacridinium salts react efficiently with aqueous alkaline hydrogen peroxide to yield the excited state of *N*-methylacridone, which luminesces with a 4 per cent quantum yield¹⁰.) The basic chemiluminescent reaction mechanism is well understood, and esters which are stable for up to one year in solution have been synthesized¹¹. The mechanism of enhanced chemiluminescent reactions is complex, and is based on the enzyme horseradish peroxidase, which catalyses the chemiluminescent oxidation of cyclic diacylhydrazines such as luminol. Luminol is oxidized by complexes formed between oxidants (hydrogen peroxide and alkali) and horseradish peroxidase, to form luminol radicals which decompose through an endoperoxide intermediate to form the excited state 3-aminophthalate dianion. When the excited state decays into its ground state, it emits light. The reaction can be improved significantly by the presence of certain phenol, naphthol, and amine "enhancers"¹².

Chemiluminescent systems such as acridium esters and luminols require hydrogen peroxide, alkali, and other reagents to generate the emitting state. The addition of these chemiluminescence-stimulating reagents generally leads to a "burst" or "flash" of light, which is difficult to record by ordinary photomultiplier tube-based instruments, leading to imprecise test results.

Flashy chemistry

Tropix has designed and synthesized a proprietary new dioxetane-based direct chemiluminescent substrate, adamantyl-1,2-dioxetane phosphate (AMPPD), for alkaline phosphatase¹³ (Fig. 1). The AMPPD molecule is stable, emits energy at the wavelength of visible light, and has a built-in energy source (its peroxy bond). The Tropix "intelligent" molecule also

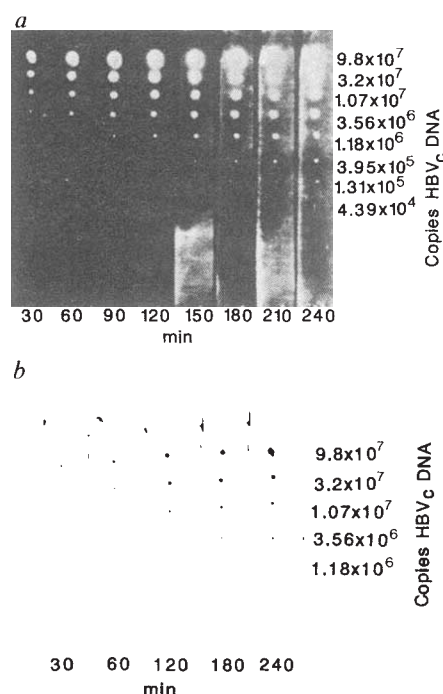


FIG. 2 a, Chemiluminescent (AMPPD) and b, colorimetric (BCIP/NBT) detection of HBVc DNA in a hybridization assay, using Polaroid Instant Black and White Type 612 film.

contains a "keyhole", specific for the alkaline phosphatase enzyme "key", which is used to unlock the stored energy of the molecule and trigger light emission. Tropix has also engineered a molecule which is activated by β -galactosidase.

Upon reaction with alkaline phosphatase, the AMPPD substrate spontaneously chemiluminesces. Unlike acridium esters and other common chemiluminescent systems, AMPPD is already in an oxygenated state, capable of releasing up to 100 kcal mol⁻¹ of energy. Alkaline phosphatase dephosphorylates AMPPD to form a dioxetane anion, which fragments into adamantanone and the excited state of methyl *meta*-oxybenzoate anion, the light emitter¹⁴.

The activation energy for AMPPD decomposition in hydrogen peroxide is 21.5 kcal mol⁻¹, as determined from Arrhenius plots. The measured decomposition parameters include both the thermal component, or the breakdown of the oxygen-oxygen bond, and the non-enzymatic hydrolysis of the protective group. In spite of its low activation energy, AMPPD is remarkably stable: the half-life of AMPPD in water is 142 hours at 30 °C, and in the presence of carbonate buffer at pH 12, it increases to 6,214 hours. AMPPD exhibits an indefinite shelf-life in the solid form at 4 °C. Because the nonenzymatic decomposition of AMPPD produces fragments which do not exhibit chemiluminescence, even in the presence of enzyme, there is no background signal¹⁴. Moreover, alkaline phosphatase-catalysed chemiluminescence from AMPPD is constant for prolonged periods of time. Using AMPPD, 0.001 attomole of alkaline phosphatase has been detected, on both immobilized membrane supports and in solution¹⁴. Comparable results have been obtained measuring the luminescent signal using a luminometer or Polaroid Type 612 film.

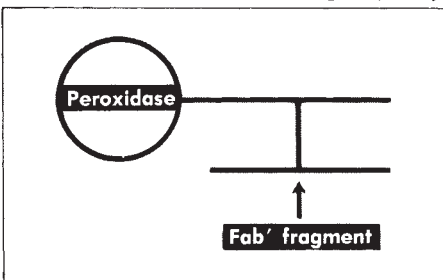
A comparison of the sensitivities of AMPPD and the chromogenic substrate 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium salt monohydrate (BCIP/NBT) in detecting alkaline phosphatase has been made in a DNA probe hybridization assay for hepatitis B virus core antigen (HBV_c) DNA (Fig. 2). The sensitivity of the assay was improved by more than two orders of magnitude using the AMPPD substrate for the alkaline phosphatase label. The use of AMPPD also reduced the time required to obtain a result from roughly 240 minutes to 30 minutes¹⁵.

AMPPD offers an alternative to colourimetric and fluorescent substrates for alkaline phosphatase. The substrate has been used in a broad range of assays, including direct assays for enzymes; immunoassays for proteins, haptens and microorganisms; and DNA probe assays¹⁶. AMPPD's strong signal and low back-

Today's immunology

A fusion protein combining the best of β -galactosidase with Protein G, a host of human cytokines and lymphokines, and a thermally regulated microplate reader are among this week's offerings for the immunologist.

Protos Laboratories is now selling a line of **peroxidase-conjugated Fab' fragments** of affinity-purified antibodies (*Reader Service No. 101*). Enzyme-labelled antibodies are being used increasingly for the localization of cellular antigens in tissue sections using light or electron microscopy. To address the demand, Protos Labs has developed goat Fab' fragments linked to peroxidase which are specific for human IgG, IgA, IgM, kappa chain and lambda chain. For use in indirect staining techniques, Protos Labs offers goat Fab' anti-mouse and anti-rabbit IgG (heavy



The Protos peroxidase-Fab' fragment hybrid. and light chains), adsorbed to insolubilized human serum. Protos Labs says its 87,000 MW Fab' conjugates penetrate tissue readily and exhibit low background staining. The Fab' conjugates cost \$80-85 (US) for 1 ml.

The **anti-phosphotyrosine fluorescein-conjugated** monoclonal antibody from Boehringer Mannheim Biochemicals is tested for its ability to stain tyrosine-phosphorylated protein in PHA-activated lymphocytes (*Reader Service No. 102*). The fluorescein conjugate supplements the company's monoclonal antibody to phosphotyrosine, which detects phos-

ground means it can be used with a variety of photosensitive detection devices, such as photomultiplier tubes, photodiodes, and photographic and X-ray films¹⁷. The utility of AMPPD in electrophoresis, blotting, sequencing, chromatography, and cytometry¹⁸ is now being tested. □

Irena Bronstein and Patricia McGrath are at Topix, Inc., 47 Wiggins Avenue, Bedford, Massachusetts 01730, USA. For more information, fill in reader service number 100.



Boehringer Mannheim's MAb's against phosphotyrosine.

phorylated tyrosine residues produced by protein kinase activities. Boehringer Mannheim says the antibody is suited for purifying and characterizing phosphotyrosyl-growth factor receptors, transforming proteins and their substrates, and that it does not cross-react with any phosphorylated amino acids, ribose phosphate or nucleotides. The antibody can also be used for Western blot analysis, immunocytochemistry and flow cytometry.

ICN Biochemicals is offering **synthetic Lipid A** for research purposes (*Reader Service No. 103*). The company says its synthetic product has identical biological and endotoxic activities in test systems for lethal toxicity, pyrogenicity, local Shwartzman activity, Limulus Amoebocyte Lysate gelation capacity, B cell mitogenicity, induction of prostaglandin synthesis in macrophages, tumour necrotizing activity and specificity of antigenic determinants as natural Lipid A. The synthetic Lipid A is available as a lyophilized powder, with no excipient added, and costs \$85 (US) for 0.1 mg.

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BRL says its **goat anti-rat IgG (H + L) antibody** and conjugates are useful for probing mouse tissues, cells or extracts with rat primary antibodies (*Reader Service No. 104*). The product is immuno-adsorbed against mouse serum to remove cross-reactivity to mouse immunoglobulins. The secondary antibodies react specifically with rat primary antibodies, and are affinity-purified using rat IgG. They are available unconjugated or conjugated to horseradish peroxidase, alkaline phosphatase or fluorescein isothiocyanate.

Cytokines and lymphokines

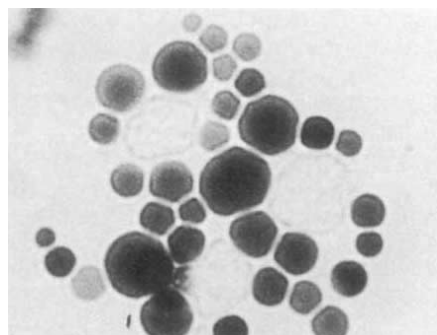
A variety of human and murine blood cell-related substances, **lymphokines and cytokines** are now available from Bachem Bioscience, Inc. (*Reader Service No. 105*). The company carries a full line of interleukins, interferons and colony-stimulating factors which are cloned, expressed, purified and tested for activity in Bachem's own molecular biology laboratories. The current list includes human interleukin-1 beta, human interleukin-2, murine interleukin-3, human interferon-gamma, and human and murine GM-CSF.

R&D Systems, Inc. sells **recombinant human cytokines** expressed, purified and assayed in the laboratories of the company from British Bio-technology's Designer Genes line of human genes (*Reader Service No. 106*). R&D Systems's range of growth factors isolated from natural sources includes bovine fibroblast growth factor alpha, bovine fibroblast growth factor beta, human and porcine tissue growth factor betas, and human and porcine platelet-derived growth factors. The company also has a line of exon-specific, anti-sense DNA probes for human interleukins, and an array of neutralizing antibodies for cytokines.

Sorting and typing

Dynal UK Ltd has a set of **immuno-magnetic polystyrene beads** for separating and quantifying subsets of human T-lymphocyte CD4 (helper-inducer) T cells, CD8 (cytotoxic-suppressor) T cells and CD2 (Pan) T cells (*Reader Service No. 107*). The M-450 Dynabeads are uniform magnetizable polystyrene beads which bind positively to the target cells when coated with specific monoclonal antibodies. Rosettes of magnetic beads form around the T cells, allowing them to be isolated in a magnetic separation step which does not involve centrifugation. The isolated cells can be quantified or used for further studies, or the supernatant — depleted of target cells — can be analysed. Dynal says the beads do not affect cell viability, and yield preparations with better than 99 per cent purity.

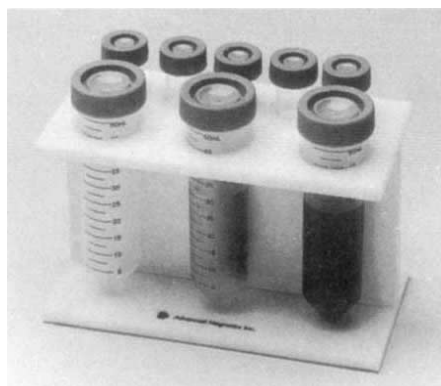
The Immunobead reagents for **human B cell labelling** from Bio-Rad consist of



Bio-Rad's Immunobead B cell labels at work.

micron-sized hydrophilic particles with covalently bound highly-purified rabbit anti-human immunoglobulin (heavy and light chains) antibodies (*Reader Service No. 108*). The Immunobead reagents simultaneously label lymphocyte surface immunoglobulins and identify any contaminating monocytes by phagocytic ingestion, says Bio-Rad. B cells are rosetted and counted using either phase or transmission microscopy, eliminating the need for fluorescent microscopy. Bio-Rad says Immunobead reagents do not react with the Fc receptors present on certain lymphocytes, so only those cells with immunoglobulin present on their surfaces are labelled. The reagent can be used simultaneously with sheep erythrocytes to rosette human B and T cells. Immunobead reagents specific for the individual human immunoglobulins IgG, IgA or IgM are available for determining subpopulations.

Magnetic cluster of differentiation (CD) antibodies for the isolation of specific leukocyte populations are now available



CD antibodies add to the usefulness of BioMag.

from Advanced Magnetix, Inc. (*Reader Service No. 109*). Antibodies for CD3, CD4, CD6, CD8, CD9, CD10, CD16, CD19 and CD25 are available conjugated to Advanced Magnetix's BioMag particles, allowing for the purification, depletion, enrichment and quantification of specific leukocytes. The company says its BioMag particles are non-toxic and biodegradable, and that shear forces on the leukocytes are minimized by the small 1 µm size of the particles.

Protein G products

The InFerGene company recently launched ProFusion β-Gal/G, a product which incorporates into one protein the active immunoglobulin-Fc binding sites of Streptococcal Protein G and the enzyme activity of *Escherichia coli* β-galactosidase (*Reader Service No. 110*). ProFusion β-Gal/G is a fusion protein created by recombinant means, and InFerGene says the product has a greater batch-to-batch consistency and a higher specific activity than similar conjugates made by chemical coupling reactions. InFerGene is now selling ProFusion β-Gal/G directly, and through its American Enzyme Corporation subsidiary.

The GammaBind G **HPLC columns** from Genex are affinity columns packed with a pH-resistant, polymer-coated silica solid support bound to recombinant protein G (*Reader Service No. 111*). Genex has engineered the protein G in the GammaBind G columns to increase its

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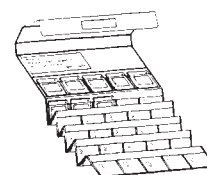
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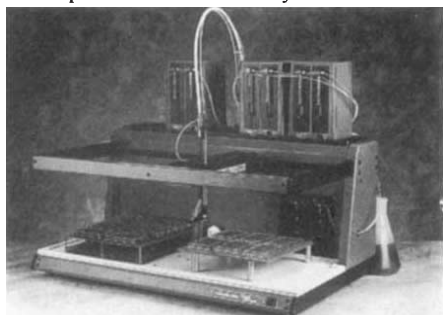
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natural resistance to proteolytic degradation. The company says the recombinant product binds specifically and only to all subtypes of IgG antibodies, with no cross reactivity with other serum proteins or IgM, IgE or IgA antibodies. The GammaBind G columns can be used for purifying or quantifying IgG antibodies in serum, ascites fluid, and hybridoma cell culture supernatants; measuring IgG levels for quality control and process monitoring; concentrating and purifying IgG monoclonal antibodies from tissue culture media; removing IgG from serum samples; and purifying mitogen-free antibodies for lymphocyte studies. The GammaBind G columns come in analytical and semi-preparative sizes, and column adaptors are available to fit them to most HPLC systems.

Harvesting and diluting

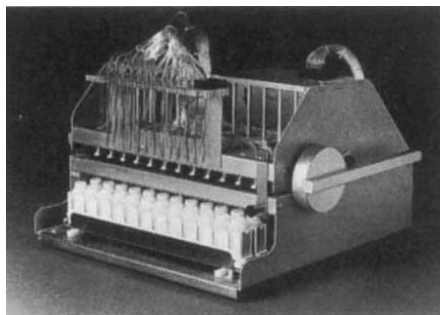
Hamilton Company has introduced a new multi-probe head accessory for its Micro-



lab 2200 **automated liquid handling station** (Reader Service No. 112). The multi-probe head utilizes up to three Microlab 941 dual-channel diluters/dispensers as well as the two internal diluter channels in the Microlab 2200. It allows spacing between probes to be varied from 9 mm up to 21 mm. Up to eight independent reagent transfers can be performed simultaneously, reducing sample preparation time, says Hamilton. The multi-probe

head can be used to add reagent to tubes or microplates by aspirating and dispensing liquid through probe needles; to add reagent from large reservoirs by pumping through the syringes and tubing; and to perform serial dilutions in microtitre plates.

Cambridge Technology, Inc. has a **sample harvester** which it says can punch, harvest and deposit samples directly into



Model 2000 PHD sample harvester from Cambridge Technology.

any type of scintillation vials (Reader Service No. 113). Cambridge Technology's model 2000 PHD sample harvester has a large-diameter collection area and the ability to handle large samples — a plus for receptor binding assays. The \$4,800 (US) model 2000 harvests 24 samples at a time onto 25 mm collection sites, and inserts them into standard test tubes, gamma counter tubes, minivials or 20-ml scintillation vials, depending on which sample tray is used. Several grades of pre-cut glass fibre filter strips are available to go with the sample harvester.

Plate readers

Molecular Devices has a **thermally regulated microplate reader** which allows researchers to perform valid kinetic anal-



The THERMOmax from Molecular Devices. uses at elevated temperatures (Reader Service No. 114). The THERMOmax from Molecular Devices controls temperature from 4 °C above ambient temperature to 42 °C, with a resolution of 0.1 °C and a temperature variation across the microplate of less than 0.5 °C, says the company. The microplate can be kept stationary for turbidimetric assays, such as clot formation assays for the detection of endotoxin, or clot dissolution assays for the detection of TPA. For assays where mixing is necessary, such as bacterial growth studies, the instrument's Automix function minimizes the settling of cells. The SOFTmax 2.0 IBM-compatible soft-

ware for the THERMOmax performs nonlinear kinetic analyses and computes time to reach V_{max} . The software also includes five standard curve algorithms and a set of displays and reports.

Millipore's new CytoFluor **fluorescent measurement system** is a computer-controlled scanning device for multiwell plates which can be used to quantify both



Less than one-minute measurements with Millipore's CytoFluor fluorescent measurement system.

soluble and cell-associated fluorescent signal, for applications in cytotoxicity testing, phenotyping, cell receptor labelling, and fluorescence-based enzyme assays (Reader Service No. 115). Millipore says the instrument reads fluorescent signal in less than one minute through standard tissue culture plastic or through membrane-based tissue culture devices. The CytoFluor system can accommodate 6-, 12-, 24- or 96-well plates, and allows multiple wavelength scanning. All operations are software-driven, and data is reported as either an EXCEL spreadsheet or a comma separated values (CSV) file for fast data analysis.

Dynatech says its MR 5000 **kinetic microplate reader** is capable of reading an entire microplate in 1.7 seconds (Reader Service No. 116). The MR 5000 has a built-



Dynatech's microplate reader for kinetic analyses.

in microprocessor capable of storing up to 50 different user-defined test protocols, and the data from 20 microplates. The integral software allows for data analysis on any of the stored data, as well as on a newly read plate. □

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